# Supporting Information

## Synthetic and immunological studies of *Salmonella* Enteritidis *O*-antigen tetrasaccharide as potential anti-*Salmonella* vaccines

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## Experimental Section

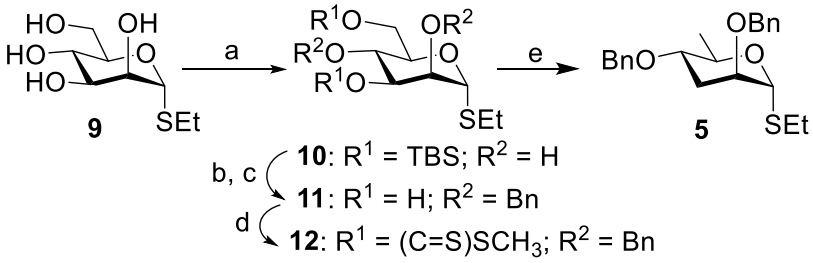
### General methods

All chemicals were reagent grade and were used as received from the manufacturer, unless otherwise noted. All reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulfate (2% $Ce(SO_4)_2$ in 2N $H_2SO_4$) sprayed plates on a hot plate. Silica gel 230-400 mesh was used for flash column chromatography. NMR spectra were recorded on Bruker Avance 500 MHz using $CDCl_3$ as solvent and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ ppm. The complete assignment of proton and carbon spectra was carried out by using a standard set of NMR experiments, e.g. $^{1}$H NMR, $^{13}$C NMR, $^{13}$C DEPT 135, 2D COSY and 2D HSQC etc. The two-dimensional NMR data were processed by Top Spin software suite (Bruker, Switzerland). MALDI-MS were recorded on a Bruker mass spectrometer. Optical rotations were recorded in a Jasco P-2000 spectrometer. For purification of Qβ, centrifugal filter units of 10, 000 and 100, 000 molecular weight cut-off (MWCO) were purchased from EMD Millipore. ESI-TOF LC-MS analysis was performed on each purified Qβ sample (1 µL, 1 mg/mL) after denaturing (Waters Xevo G2-XS UPLC/MS/MS). MALDI-TOF MS analysis was performed on each purified BSA sample (10 µL, 1 mg/mL) after denaturing and desalting the sample using Cleanup C18 Pipette Tips (Agilent Technologies). The mixture (0.6 µL) and matrix solution (0.6 µL, 10 mg/mL sinapic acid in 50/50/0.1 $CH_3CN/H_2O$/TFA was spotted on a MALDI plate, air-dried, and analysed by MALDI-TOF mass spectrometry (Applied Biosystems Voyager DE STR). Protein concentration was measured using the Coomassie Plus Protein Reagent (Bradford Assay, Pierce) with bovine serum albumin (BSA) as the standard.

**Characterization of anomeric stereochemistry.** The stereochemistries of the newly formed glycosidic linkages were determined by $^{3}J_{H1,H1}$ through $^{1}$H-NMR and/or $^{1}J_{C1,H1}$ through gHMQC 2-D NMR (without $^{1}$H decoupling). For the galactoside linkages, smaller coupling constants of $^{3}J_{H1,H2}$ (around 3 Hz) indicate α linkages and larger coupling constants $^{3}J_{H1,H1}$ (7.2 Hz or larger) indicate β linkages. For all glycosyl linkages including mannoside, rhamnoside and tyveloside, $^{1}J_{C1,H1}$ around 170 Hz suggests α linkages and 160 Hz suggests β linkages.[1]

**Phenyl 2-*O*-acetyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (6):** To a solution of compound **13** (5 g, 11.0 mmol)[2] in DMF (30 ml) were added triethylorthoacetate (3 ml, 16.5 mmol) and *p*-TSA (100 mg). The mixture was stirred for 3 h at room temperature. The reaction was quenched with $Et_3N$ (1 ml) and the organic solvent was evaporated under reduced pressure. The residue was purified over $SiO_2$ column chromatography using hexane-EtOAc (5:1) as the eluant to give pure compound **6** (4.9 g, 90%). White solid; m.p. 78-80 °C [EtOH]; $[\alpha]_D^{25}$ +127 (*c* 1.0, $CHCl_3$); IR (KBr): 3571, 3058, 2828, 1744, 1374, 1234, 1073, 1026, 745, 696 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.46-7.21 (m, 15 H, Ar-*H*), 5.50 (d, *J* = 1.5 Hz, 1 H, H-1), 5.32 (dd, *J* = 3.0, 1.5 Hz, 1 H, H-2), 4.78 (d, *J* = 11.0 Hz, 1 H, PhC*H*), 4.67 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.60 (d, *J* = 11.0 Hz, 1 H, PhC*H*), 4.48 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.28-4.25 (m, 1 H, H-5), 4.07 (dd, *J* = 9.0, 3.5 Hz, 1 H, H-3), 3.85-3.81 (m, 2 H, H-4, H-$6_a$), 3.70 (dd, *J* = 11.0, 2.0 Hz, 1 H, H-$6_b$), 2.13 (s, 3 H, COC*H*$_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 170.4 (*C*O$CH_3$), 138.1-127.6 (Ar-*C*), 86.0 (C-1), 76.0 (C-4), 74.9 (Ph*C*$H_2$), 74.0 (C-2), 73.4 (Ph*C*$H_2$), 72.2 (C-5), 70.9 (C-3), 68.8 (C-6), 21.0 (*C*O$CH_3$); HRMS calcd. for $C_{28}H_{30}O_6S$ (494.17): $[M+NH_4]^+$ 512.2101; found: 512.2101.

Reagents and conditions: (a) TBSCl, imidazole, DMF, room temperature, 12 h, 65%; (b) NaH, BnBr, DMF, room temperature, 1 h, 94%; (c) cat. p-TSA, CH3OH, room temperature, 91%; (d) NaH, imidazole, $CS_2$, THF then $CH_3I$, room temperature, 87%; (e) tri-n-butyl tin hydride, AIBN, toluene, reflux, 3 h, 45 %;




**Ethyl 3,6-di-*O*-(*tert*-butyldimethylsilyl)-1-thio-α-D-mannopyranoside (10):** To a solution of compound **9** (6.2 g, 27.6 mmol)[3] in DMF (100 ml) were added imidazole (4.7 g, 69.1 mmol) and TBSCl (10.3 g, 69.1 mol) at 0 °C and the reaction mixture was stirred at room temperature for 12 h and then quenched with $CH_3OH$. The mixture was diluted with EtOAc (200 ml) and washed with 1M HCl, satd. $NaHCO_3$ and brine. The organic layer was separated, dried with $Na_2SO_4$, filtered, and evaporated to dryness. The crude product was purified by $SiO_2$ flash column chromatography using petroleum ether-$CH_2Cl_2$ (6:1) as the eluant to give pure compound **10** (8.13 g, 65%).

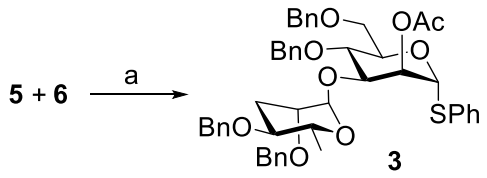
Colorless oil; $[\alpha]_D^{25}$ +108 (*c* 1.0, $CHCl_3$); IR (neat): 3415, 2929, 2853, 1649, 1471, 1361, 1255, 1096, 1050 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 5.07 (s, 1 H, H-1), 3.92 (m, 1 H, H-2), 3.80-3.74 (m, 3 H, H-3, H-4, H-5), 3.66-3.58 (m, 2 H, H-$6_{ab}$), 2.69 (br s, 1 H, O*H*), 2.59-2.46 (m, 2 H, SC*H*$_2$CH$_3$), 1.98 (br s, 1 H, O*H*), 1.23 (s, 3 H, SCH$_2$C*H*$_3$), 0.84, 0.83 (2 s, 18 H, Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.04, 0.03, 0.02, 0.01 (4 s, 12 H, Si(C*H*$_3$)$_2$C(CH$_3$)$_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 84.6 (C-1), 73.1 (C-5), 72.3 (C-3), 71.7 (C-2), 70.3 (C-4), 63.9 (C-6), 25.9, 25.7 (6 C, Si(CH$_3$)$_2$C(*C*H$_3$)$_3$), 24.7 (S*C*H$_2$CH$_3$), 18.3, 18.1 (2 C, Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), 14.8 (SCH$_2$*C*H$_3$), −4.5, −4.7, −5.3, −5.4 (4 C, Si(*C*H$_3$)$_2$C(CH$_3$)$_3$); HRMS calcd. for $C_{20}H_{24}O_5SSi_2$ (452.24): $[M+Na]^+$ 475.2340; found: 475.2349.

**Ethyl 2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (11)**: To a solution of compound **10** (8 g, 17.6 mmol) in DMF (80 ml) were added BnBr (4.75 ml, 38.9 mmol) and NaH (2 g, 84.9 mmol) at 0 °C and the reaction mixture was stirred for 1 h and quenched with $CH_3OH$ (1 mL). The reaction mixture was diluted with $Et_2O$ (200 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. To a solution of the crude product in $CH_3OH$ (50 ml) was added *p*-TSA (200 mg) and the mixture was stirred for 2 h and quenched with $Et_3N$ (3 ml). The mixture was evaporated and the crude product was purified by flash column chromatography with hexane-EtOAc (3:1) as the eluant to give pure compound **11** (6 g, 85%). White solid; m.p. 71-73 °C [EtOH]; $[\alpha]_D^{25}$ +104 (*c* 1.0, $CHCl_3$); IR (KBr): 3442, 2922, 2872, 1495, 1454, 1350, 1268, 1206, 1090 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.35-7.22 (m, 10 H, Ar-*H*), 5.31 (s, 1 H, H-1), 4.87 (d, *J* = 11.0 Hz, 1 H, PhC*H*$_2$), 4.69 (d, *J* = 11.5 Hz, 1 H, PhC*H*$_2$), 4.64 (d, *J* = 11.0 Hz, 1 H, PhC*H*$_2$), 4.57 (d, *J* = 12.0 Hz, 1 H, PhC*H*$_2$), 3.95-3.89 (m, 2 H, H-3, H-5), 3.79-3.76 (m, 3 H, H-2, H-$6_{ab}$), 3.72 (t, *J* = 9.5 Hz each, 1 H, H-4), 2.59-2.44 (m, 3 H, SC*H*$_2$CH$_3$, O*H*), 2.20 (br s, 1 H, O*H*), 1.24 (t, *J* = 7.5 Hz each, 3 H, SCH$_2$C*H*$_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 138.4-127.7 (Ar-*C*), 81.3 (C-1), 80.0 (C-2), 76.5 (C-4), 74.8 (Ph*C*H$_2$), 72.6 (Ph*C*H$_2$), 72.2 (C-3), 71.8 (C-5), 62.6 (C-6), 25.1 (S*C*H$_2$CH$_3$), 14.8 (SCH$_2$*C*H$_3$); HRMS calcd. for $C_{22}H_{28}O_5S$ (404.16): $[M+Na]^+$ 427.1549; found: 427.1550.

**Ethyl 2,4-di-*O*-benzyl-3,6-di-*O*-(methylthio)thiocarbonyl-1-thio-α-D-mannopyranoside (12):** To a solution of compound **11** (6 g, 14.8 mmol) in anhydrous THF (90 ml) were added NaH (1.56 g, 65.3 mmol) and imidazole (1 g, 14.8 mmol). After stirring the reaction mixture for 1.5 h, $CS_2$ (3.7 ml, 59.4 mmol) was added and it was further stirred at room temperature for 3.5 h. Then $CH_3I$

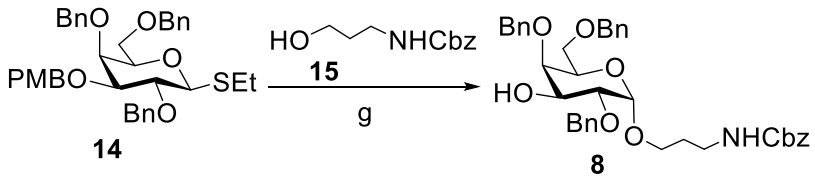
(3.7 ml, 59.4 mmol) was added and keeping the stirring continued for another 1.5 h. The mixture was ice-cooled and quenched with $CH_3OH$ (5 ml) and evaporated to dryness. The residue was dissolved in EtOAc (500 ml) and successively washed with 1N HCl (200 ml), satd. $NaHCO_3$ (200 ml) and brine (200 ml). The organic phase was dried over $Na_2SO_4$, evaporated and the crude product was passed through a short pad of $SiO_2$ to give pure compound **12** (7.5 g, 87%). White solid; m.p. 92-94 ºC; $[\alpha]_D^{25}$ +65 (*c* 1.0, $CHCl_3$); IR (KBr): 2925, 2880, 1492, 1452, 1204, 1067, 755, 698 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.34-7.24 (m, 10 H, Ar-*H*), 6.01 (dd, *J* = 9.0, 3.0 Hz, H-3), 5.32 (s, 1 H, H-1), 4.77-4.72 (m, 3 H, PhC*H*, H-$6_{ab}$), 4.62-4.51 (m, 3 H, PhC*H*), 4.40 (m, 1 H, H-5), 4.25 (m, 1 H, H-2), 4.21 (t, *J* = 9.5 Hz each, 1 H, H-4), 2.63 (m, 2 H, SC*H*$_2$CH$_3$), 2.59, 2.54 (2 s, 6 H, CSSC*H*$_3$), 1.28 (t, *J* = 6.5 Hz each, 3 H, SCH$_2$C*H*$_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 214.9, 214.7 (2 C, *C*SSCH$_3$), 137.5-127.5 (Ar-*C*), 83.1 (C-3), 82.0 (C-1), 76.4 (C-2), 74.8 (Ph*C*H$_2$), 72.8 (C-4), 72.7 (Ph*C*H$_2$), 72.0 (C-6), 69.7 (C-5), 24.8 (S*C*H$_2$CH$_3$), 19.3, 18.7 (CSS*C*H$_3$), 14.8 (SCH$_2$*C*H$_3$); HRMS calcd. for $C_{26}H_{32}O_5S_5$ (584.08): $[M+H]^+$ 585.0926; found: 585.0939.

**Ethyl 2,4-di-*O*-benzyl-3,6-dideoxy-1-thio-α-D-*arabino*-hexopyranoside (5):** To a solution of compound **12** (5 g, 8.5 mmol) in anhydrous toluene (50 ml) were added AIBN (100 mg, 0.5 mmol) and tributyltin hydride (5.9 ml, 20.5 mmol) and the reaction mixture was stirred at reflux condition for 3 h. The reaction mixture was concentrated under reduced pressure and purified over $SiO_2$ column chromatography using hexane-EtOAc (10:1) as the eluant to give pure compound **5** (1.4 g, 45% yield). Colorless oil; $[\alpha]_D^{25}$ +134 (*c* 1.0, $CHCl_3$); IR (neat): 2971, 2925, 2869, 1495, 1452, 1365, 1262, 1204, 1098 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.35-7.25 (m, 10 H, Ar-*H*), 5.22 (s, 1 H, H-1), 4.61 (d, *J* = 12.5 Hz, 1 H, PhC*H*$_2$), 4.58 (d, *J* = 11.5 Hz, 1 H, PhC*H*$_2$), 4.48 (d, *J* = 12.0 Hz, 1 H, PhC*H*$_2$), 4.46 (d, *J* = 11.5 Hz, 1 H, PhC*H*$_2$), 4.06 (m, 1 H, H-5), 3.69 (br s, 1 H, H-2), 3.50 (m, 1 H, H-4), 2.66 (m, 1 H, SC*H*$_2$CH$_3$), 2.24 (m, 1 H, H-$3_a$), 1.74 (m, 1 H, H-$3_b$), 1.32 (t, *J* = 7.0 Hz each, 3 H, SCH$_2$C*H*$_3$), 1.30 (d, *J* = 6.0 Hz, 3 H, CC*H*$_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 138.5-127.5 (Ar-*C*), 81.7 (C-1), 76.6 (C-2), 75.7 (C-4), 71.1 (Ph*C*H$_2$), 70.8 (Ph*C*H$_2$), 68.1 (C-5), 31.0 (C-3), 24.9 (S*C*H$_2$CH$_3$), 18.0 (SCH$_2$*C*H$_3$), 15.1 (C*C*H$_3$); HRMS calcd. for $C_{22}H_{28}O_3S$ (372.17): $[M+Na]^+$ 395.1651; found: 395.1640.



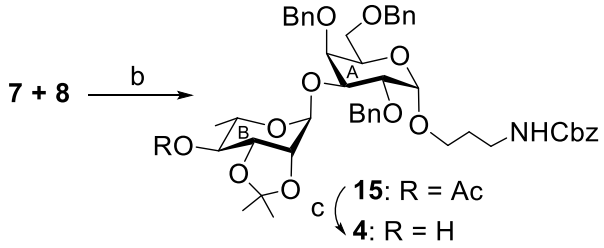

(a) NIS, TMSOTf, $CH_2Cl_2$, 20 °C, 45 min, 85%;

**Phenyl (2,4-di-*O*-benzyl-3,6-dideoxy-1-thio-α-D-*arabino*-hexopyranosyl)-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (3):** To a solution of compound **5** (1.0 g, 2.68 mmol) and compound **6** (1.1 g, 2.22 mmol) in anhydrous $CH_2Cl_2$-$Et_2O$ (20 ml; 1:1) was added MS 4Å (1 g) and the reaction mixture was stirred at room temperature for 45 min and then cooled to −20 °C under argon. To the cooled reaction mixture was added NIS (665 mg, 2.97 mmol) followed by TMSOTf (30 µl) and it was allowed to stir at −20 °C for 45 min. The reaction mixture was filtered through a Celite bed® and washed with $CH_2Cl_2$ (100 ml). The organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$ and water in succession, dried ($Na_2SO_4$), and concentrated. The crude product was purified over $SiO_2$ using hexane-EtOAc (5:1) as the eluant to give pure compound **3** (1.5 g, 85%). Colorless oil; $[\alpha]_D^{25}$ +39 (*c* 1.0, $CHCl_3$); IR (neat): 3032, 2922, 2853, 1742, 1495, 1454, 1365, 1238, 1219, 1137, 1048 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 77.4-7.14 (m, 25 H, Ar-*H*), 5.52 (d, *J* = 1.0 Hz, 1 H, H-$1_C$), 5.37-5.36 (m, 1 H, H-$2_C$), 4.96 (br s, 1 H, H-$1_D$), 4.47-4.36 (m, 7 H, PhC$H_2$), 4.28-4.26 (m, 2 H, H-$5_C$, PhC$H_2$), 4.13 (dd, *J* = 9.5, 3.0 Hz, 1 H, H-$3_C$), 3.97 (t, *J* = 10.0, 9.5 Hz, 1 H, H-$4_C$), 3.84 (dd, *J* = 11.0, 5.5 Hz, 1 H, H-$6_{bC}$), 3.70-3.63 (m, 2 H, H-$5_D$, H-$6_{aC}$), 3.53 (br s, 1 H, H-$2_D$), 3.50-3.48 (m, 1 H, H-$4_D$), 2.13-2.10 (m, 4 H, H-$3_{aD}$, COC$H_3$), 1.72-1.68 (m, 1 H, H-$3_{bD}$), 1.27 (d, *J* = 6.0 Hz, CC$H_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 169.9 (*C*O$CH_3$), 138.5-127.2 (Ar-*C*), 99.3 (C-$1_D$), 85.8 (C-$1_C$), 77.6 (C-$3_C$), 75.3 (C-$4_D$), 75.1 (C-$2_D$), 74.8 (Ph*C*$H_2$), 74.8 (C-$4_C$), 73.9 (C-$2_C$), 73.4 (Ph*C*$H_2$), 72.6 (C-$5_C$), 71.2 (Ph*C*$H_2$), 70.8 (Ph*C*$H_2$), 69.2 (C-$5_D$), 68.7 (C-$6_C$), 29.7 (C-$3_D$), 21.0 (CO*C*$H_3$), 18.0 (C*C*$H_3$); HRMS calcd. for $C_{48}H_{52}O_9S$ (804.33): $[M+NH_4]^+$ 822.3670; found: 822.3706.




(g) NIS, TfOH, $CH_2Cl_2$:$Et_2O$ (1:3), 20 °C, 0.5 h, 65%.

**3-(*N*-Benzyloxycarbonyl)aminopropyl 2,4,6-tri-*O*-benzyl-α-D-galactopyranoside (8):** To a solution of compound **14** (4 g, 6.51 mmol) and 3-(benzyloxycarbonyl)amino propanol **15** (2.26 g, 7.81 mmol) in anhydrous $CH_2Cl_2$-$Et_2O$ (100 ml; 1:3) was added MS 4Å (4 g) and the reaction mixture was cooled to −20 °C under argon. To the cooled reaction mixture was added NIS (1.61 g, 7.16 mmol) followed by TfOH (80 µl) and it was allowed to stir at −20 °C for 30 min. Then the reaction mixture was allowed to stir at 0 °C for 1 h, filtered through a Celite bed® and washed with $CH_2Cl_2$ (100 ml). The organic layer was washed with 5% aq. $Na_2S_2O_3$ (50 mL), satd. $NaHCO_3$ (50 mL) and water (50 mL) in succession, dried ($Na_2SO_4$), and concentrated. The crude product was purified over $SiO_2$ using hexane-EtOAc (2:1) as eluant to give pure compound **8** (2.71 g, 65%). Colorless oil; $[\alpha]_D^{25}$ +98 (*c* 1.0, $CHCl_3$); IR (neat): 3351, 3032, 2922, 2872, 1704, 1522, 1454, 1342, 1249, 1092, 1027 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.29-7.20 (m, 20 H, Ar-*H*), 5.66 (br s, 1 H, N*H*), 5.03 (br s, 2 H, COOC$H_2$Ph), 4.81 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.71 (d, *J* = 3.0 Hz, 1 H, H-1), 4.67-4.54 (m, 3 H, PhC*H*), 4.48 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.39 ( d, *J* = 12.0 Hz, 1 H, PhC*H*), 3.99 (dd, *J* = 2.5, 10.0 Hz, 1 H, H-3), 3.86 (t, *J* = 6.0, 6.5 Hz, 1 H, H-5), 3.80-3.73 (m, 3 H, H-2, H-4, O$CH_2$), 3.47-3.41 (m, 3 H, H-$6_b$, OC$H_2$, NC$H_2$), 3.37-3.35 (m, 1 H, H-$6_a$), 3.19-3.16 (m, 1 H, NC$H_2$), 1.77-175 (m, 2 H, $CH_2CH_2CH_2$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 156.1 (*C*OOCH$_2$Ph), 138.1-127.5 (Ar-*C*), 97.1 (C-1), 77.4 (C-2), 76.4 (C-3), 74.8 (Ph*C*H$_2$), 73.3 (Ph*C*H$_2$), 73.0 (O*C*H$_2$), 70.4 (C-4), 69.7 (C-5), 69.2 (Ph*C*H$_2$), 66.7 (C-6), 66.2 (COO*C*H$_2$Ph), 39.1 (N*C*H$_2$), 29.1 ($CH_2CH_2CH_2$); HRMS calcd. for $C_{38}H_{43}NO_8$ (641.29): $[M+Na]^+$ 664.2881; found: 664.2911. The α-configuration of the newly formed glycosidic linkage in **8** was confirmed by the coupling constant ($J_{1,2}$) between the H-1 and H-2 of the hexose [δ 4.71 (d, *J* = 3.0 Hz, 1 H, H-1)].



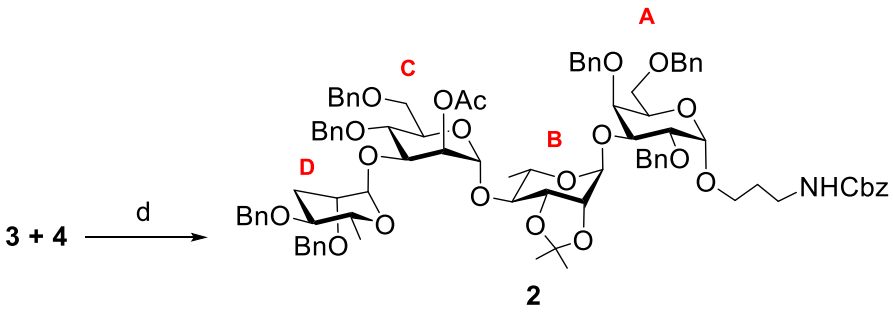

(b) NIS, TMSOTf, $CH_2Cl_2$, 30 °C, 25 min, 82%;
(c) $NaOCH_3$, $CH_3OH$, room temperature, 1 h, 95%;

**3-(*N*-Benzyloxycarbonyl)aminopropyl (4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamno-pyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-galactopyranoside (15):** To a solution of compound

**7** (545 mg, 1.87 mmol) and compound **8** (1 g, 1.56 mmol) in anhydrous $CH_2Cl_2$ (15 ml) was added MS 4Å (1.5 g) and the reaction mixture was cooled to −30 °C under argon. To the cooled reaction mixture was added NIS (510 mg, 2.26 mmol) followed by TMSOTf (20 µl) and it was allowed to stir at −30 °C for 25 min. The reaction mixture was filtered through a Celite® bed and washed with $CH_2Cl_2$ (50 ml). The organic layer was washed with 5% aq. $Na_2S_2O_3$ (50 mL), satd. $NaHCO_3$ (50 mL) and water (50 mL) in succession, dried ($Na_2SO_4$) and concentrated. The crude product was purified over $SiO_2$ using hexane-EtOAc (3:1) as eluant to give pure compound **15** (1.1 g, 82%). Colorless oil; $[\alpha]_D^{25}$ +33 (*c* 1.0, $CHCl_3$); IR (neat): 2982, 2933, 1740, 1374, 1721, 1522, 1454, 1374, 1234, 1090, 1043 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.32-7.22 (m, 20 H, Ar-*H*), 5.87 (br s, 1 H, N*H*), 5.43 (s, 1 H, H-$1_B$), 5.06-4.98 (m, 2 H, COOC$H_2$Ph), 4.83-4.76 (m, 3 H, H-$4_B$, PhC*H*), 4.74 (d, *J* = 3.0 Hz, 1 H, H-$1_A$), 4.63 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.56-4.53 (m, 3 H, PhC*H*), 4.50 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.41 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.13-4.09 (m, 2 H, H-$2_B$, H-$3_A$), 4.00-3.90 (m, 3 H, H-$3_B$, H-$2_A$, H-$5_A$), 3.84-3.82 (m, 1 H, OC$H_2$), 3.74 (d, *J* = 1.4 Hz, H-$4_A$), 3.70-3.66 (m, 1 H, H-$5_B$), 3.48-3.44 (m, 3 H, H-$6_{abA}$, NC$H_2$), 3.36-3.34 (m, 1 H, OC$H_2$), 3.09-3.08 (m, 1 H, NC$H_2$), 2.08 (s, 3 H, COC$H_3$), 1.80-1.70 (m, 2 H, $CH_2CH_2CH_2$), 1.51, 1.29 (2 s, 6 H, C(C$H_3$)$_3$), 1.12 (d, *J* = 6.5 Hz, 3 H, CC$H_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 169.6 (*C*OCH$_3$), 156.3 (*C*OOCH$_2$Ph), 138.4-127.7 (Ar-*C*), 109.6 (*C*(CH$_3$)$_3$), 98.8 (C-$1_B$), 97.5 (C-$1_A$), 77.4 (C-$4_A$), 76.8 (C-$2_B$), 76.0 (2 C, C-$2_A$, C-$5_A$), 75.5 (C-$3_A$), 74.8 (Ph*C*H$_2$), 74.4 (C-$4_B$), 73.4 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 69.7 (C-$3_B$), 68.7 (C-$6_A$), 67.7 (O*C*H$_2$), 66.1 (COO*C*H$_2$Ph), 64.6 (C-$5_B$), 39.6 (N*C*H$_2$), 29.0 ($CH_2CH_2CH_2$), 27.7, 26.5 (2 C, C(*C*H$_3$)$_3$), 21.0 (CO*C*H$_3$), 16.9 (C*C*H$_3$); HRMS calcd. for $C_{49}H_{59}NO_{13}$ (869.39): $[M+H]^+$ 870.4059; found: 870.4095.

**3-(*N*-Benzyloxycarbonyl)aminopropyl (2,3-*O*-isopropylidene-α-L-rhamnopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-galactopyranoside (4):** A solution of compound **15** (1 g, 1.15 mmol) in 0.1 M $CH_3ONa$ in $CH_3OH$ (30 ml) was allowed to stir at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W-X8 ($H^+$) resin, filtered and concentrated. The crude product was passed through a small pad of $SiO_2$ using hexane-EtOAc (3:2) as the eluant to give pure compound **4** (900 mg, 95%). Colorless oil; $[\alpha]_D^{25}$ +26 (*c* 1.0, $CHCl_3$); IR (neat): 3407, 2929, 1700, 1524, 1454, 1259, 1213, 1027, 745, 696 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.32-7.21 (m, 20 H, Ar-*H*), 5.88 (br s, 1 H, N*H*), 5.39 (br s, 1 H, H-$1_B$), 5.07-4.98 (m, 2 H, COOC$H_2$Ph), 4.80 (d, *J* = 12.0 Hz, PhC*H*), 4.74 (d, *J* = 4.0 Hz, 1 H, H-$1_A$), 4.64 (d, *J* = 12.0 Hz, 1 H, PhC*H*),

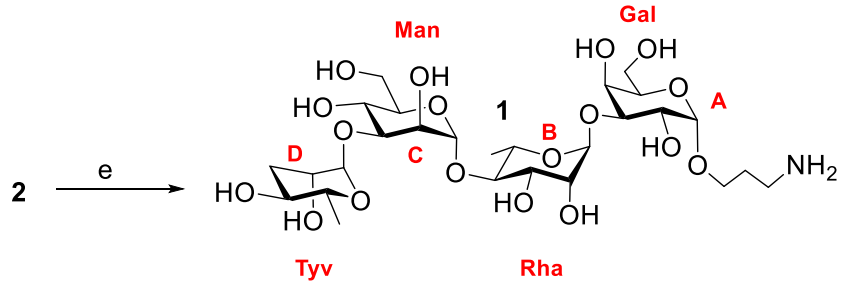
4.56-4.46 (m, 3 H, PhC*H*), 4.40 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.14-4.10 (m, 2 H, H-$2_B$, H-$3_A$), 3.97-3.89 (m, 3 H, H-$3_B$, H-$2_A$, H-$5_A$), 3.84-3.82 (m, 1 H, OC$H_2$), 3.76 (d, *J* = 2.5 Hz, 1 H, H-$4_A$), 3.66-3.63 (m, 1 H, H-$5_B$), 3.47-3.40 (m, 3 H, H-$6_{abA}$, NC$H_2$), 3.37-3.33 (m, 2 H, OC$H_2$, H-$4_B$), 3.10-3.09 (m, 1 H, NC$H_2$), 2.50 (br s, 1 H, O*H*), 1.73 (m, 2 H, $CH_2CH_2CH_2$), 1.30, 1.27 (2 C, C(C$H_3$)$_3$), 1.25 (d, *J* = 6.5 Hz, 3 H, CC$H_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 156.4 (*C*OOCH$_2$Ph), 138.4-127.6 (Ar-*C*), 109.3 (*C*(CH$_3$)$_3$), 98.9 (C-$1_B$), 97.5 (C-$1_A$), 79.2 (C-$4_A$), 77.5 (C-$5_A$), 76.8 (C-$2_A$), 76.0 (C-$2_B$), 75.9 (C-$3_A$), 74.8 (Ph*C*H$_2$), 74.6 (C-$4_B$), 73.3 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 69.9 (C-$3_B$), 68.9 (C-$6_A$), 67.7 (O*C*H$_2$), 66.3 (C-$5_B$), 66.1 (COO*C*H$_2$Ph), 39.6 (N*C*H$_2$), 29.0 ($CH_2CH_2CH_2$), 28.1, 26.3 (2 C, C(*C*H$_3$)$_3$), 17.3 (C*C*H$_3$); HRMS calcd. for $C_{47}H_{57}NO_{12}$ (827.38): $[M+Na]^+$ 850.3773; found: 850.3810.




(d) NIS, TMSOTf, $CH_2Cl_2$, -15 °C, 40 min, 78%

**3-(*N*-Benzyloxycarbonyl)aminopropyl (2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-(1→3)-(2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2,3-*O*-isopropylidene-α-L-rhamnopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-galactopyranoside (2):** To a solution of compound **3** (585 mg, 0.72 mmol) and compound **4** (500 mg, 0.60 mmol) in anhydrous $CH_2Cl_2$ (12 ml) was added MS 4Å (1 g) and the reaction mixture was cooled to −15 °C under argon. To the cooled reaction mixture was added NIS (180 mg, 0.79 mmol) followed by TMSOTf (10 µl) and it was allowed to stir at −15 °C for 40 min. The reaction mixture was filtered through a Celite® bed and washed with $CH_2Cl_2$ (50 ml). The organic layer was washed with 5% aq. $Na_2S_2O_3$ (50 mL), satd. $NaHCO_3$ (50 mL), and water (50 mL) in succession, dried ($Na_2SO_4$) and concentrated. The crude product was purified over $SiO_2$ using hexane-EtOAc (3:1) as the eluant to give pure compound **2** (700 mg, 78%). Colorless oil; $[\alpha]_D^{25}$ +28 (*c* 1.0, $CHCl_3$); IR (neat): 3032, 2929, 2865, 1740, 1719, 1497, 1372, 1240, 1137, 1090, 982 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): δ 7.76-7.12 (m, 40 H, Ar-*H*), 5.88 (br s, 1 H, N*H*), 5.40 (br s, 1 H, H-$1_B$), 5.14 (br s, 1 H,

H-2$_C$), 5.03-5.02 (m, 2 H, COOC*H*$_2$Ph), 4.99 (br s, 1 H, H-1$_C$), 4.96 (br s, 1 H, H-1$_D$), 4.80 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.77 (d, *J* = 3.0 Hz, 1 H, H-1$_A$), 4.71-4.43 (m, 11 H, PhC*H*), 4.38 (d, *J* = 12.5 Hz, 1 H, PhC*H*), 4.29 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.14-4.10 (m, 3 H, H-2$_B$, H-3$_A$, H-3$_C$), 4.05-4.04 (m, 2 H, H-5$_A$, H-5$_C$), 3.99 (dd, *J* = 9.0, 6.0 Hz, 1 H, H-2$_A$), 3.93-3.92 (m, 2 H, H-3$_B$, H-4$_C$), 3.85-3.83 (m, 2 H, H-6$_{aC}$, OC*H*), 3.76-3.68 (m, 2 H, H-4$_A$, H-5$_B$), 3.63-3.61 (m, 2 H, H-5$_D$, H-6$_{bC}$), 3.56 (br s, 1 H, H-2$_D$), 3.50-3.34 (m, 6 H, H-4$_B$, H-4$_D$, H-6$_{abA}$, NC*H*, OC*H*), 3.15-3.10 (m, 1 H, NC*H*), 2.19-2.16 (m, 1 H, H-3$_{aD}$), 2.14 (s, 3 H, COC*H*$_3$), 1.76-1.69 (m, 4 H, H-3$_{bD}$, CH$_2$C*H*$_2$CH$_2$), 1.43, 1.27 (2 s, 6 H, C(C*H*$_3$)$_2$), 1.24, 1.21 (m, 6 H, 2 CC*H*$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 170.7 (*C*OCH$_3$), 156.5 (*C*OOCH$_2$Ph), 138.5-127.3 (Ar-*C*), 108.9 (*C*(CH$_3$)$_2$, 99.5 (C-1$_D$), 98.7 (C-1$_B$), 97.9 (C-1$_C$), 97.5 (C-1$_A$), 80.6 (C-4$_D$), 77.8 (C-4$_A$), 77.3 (C-3$_C$), 76.8 (C-2$_A$), 76.6 (C-4$_C$), 76.1 (C-3$_A$), 75.8 (C-2$_D$), 75.5 (C-4$_B$), 75.1 (C-2$_B$), 74.9 (PhCH$_2$), 74.8 (PhCH$_2$), 74.1 (C-5$_A$), 73.5 (PhCH$_2$), 73.4 (PhCH$_2$), 72.9 (PhCH$_2$), 72.4 (C-2$_C$), 71.3 (C-5$_C$), 71.2 (PhCH$_2$), 70.8 (PhCH$_2$), 70.0 (C-3$_B$), 69.1 (C-5$_B$), 69.0 (C-6$_A$), 68.3 (C-6$_C$), 67.6 (OCH$_2$), 66.2 (COOCH$_2$Ph), 65.6 (C-5$_D$), 39.5 (NCH$_2$), 29.7 (CH$_2$CH$_2$CH$_2$), 29.5 (C-3$_D$), 28.9, 28.1 (2 C, C(CH$_3$)$_3$), 21.0 (COCH$_3$), 18.0, 17.2 (2 C, CCH$_3$); HRMS calcd. for $C_{89}H_{103}NO_{21}$ (1521.70): $[M+NH_4]^+$ 1539.7361; found: 1539.7435. The α-glycosidic linkages of compound **2** was confirmed by the $J_{1,2}$ coupling constant and $J_{C1/H1}$ in $^1$H coupled $^{13}$C NMR spectra of the corresponding hexose moieties [δ 5.40 (br s, 1 H, H-1$_B$), 4.77 (d, *J* = 3.0 Hz, 1 H, H-1$_A$), 4.99 (br s, 1 H, H-1$_C$), 4.96 (br s, 1 H, H-1$_D$) in the $^1$H NMR and δ 99.5 ($J_{C1/H1}$ = 167.1 Hz, C-1$_D$), 98.7 ($J_{C1/H1}$ = 169.6 Hz, C-1$_B$), 97.9 ($J_{C1/H1}$ = 175.2 Hz, C-1$_C$), 97.5 ($J_{C1/H1}$ = 166.4 Hz, C-1$_A$), in the $^{13}$C-NMR spectrum].

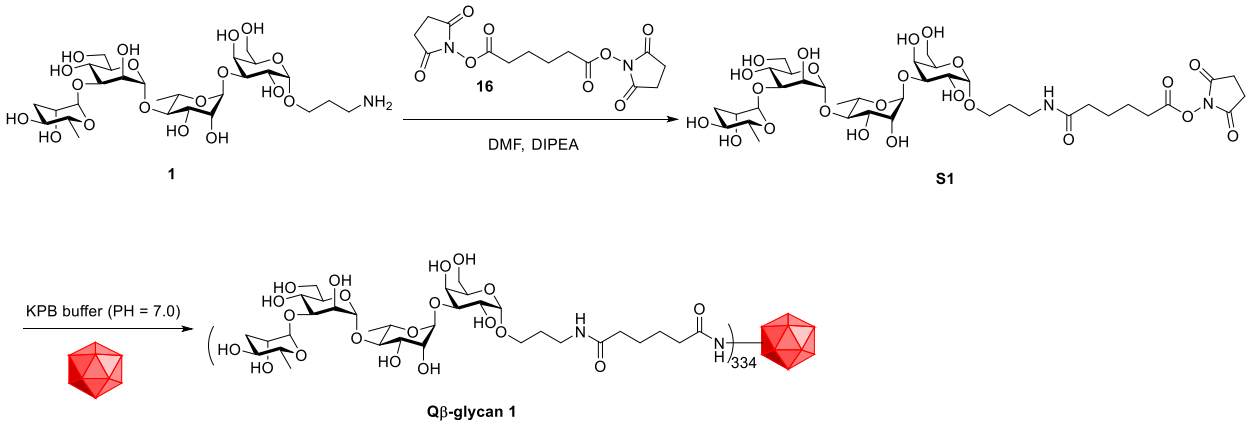



(e) (i) NaOCH$_3$, CH$_3$OH, room temperature, 2 h (ii) 80% AcOH, 80 °C, 2 h; (iii) 20% Pd(OH)$_2$-C, H$_2$, CH$_3$OH, room temperature, 16 h, 57% for three steps.

**3-Aminopropyl (3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-(1→3)-(α-D-mannopyranosyl)-(1→4)-(α-L-rhamnopyranosyl)-(1→3)-α-D-galactopyranoside (1):** A solution of compound **2**

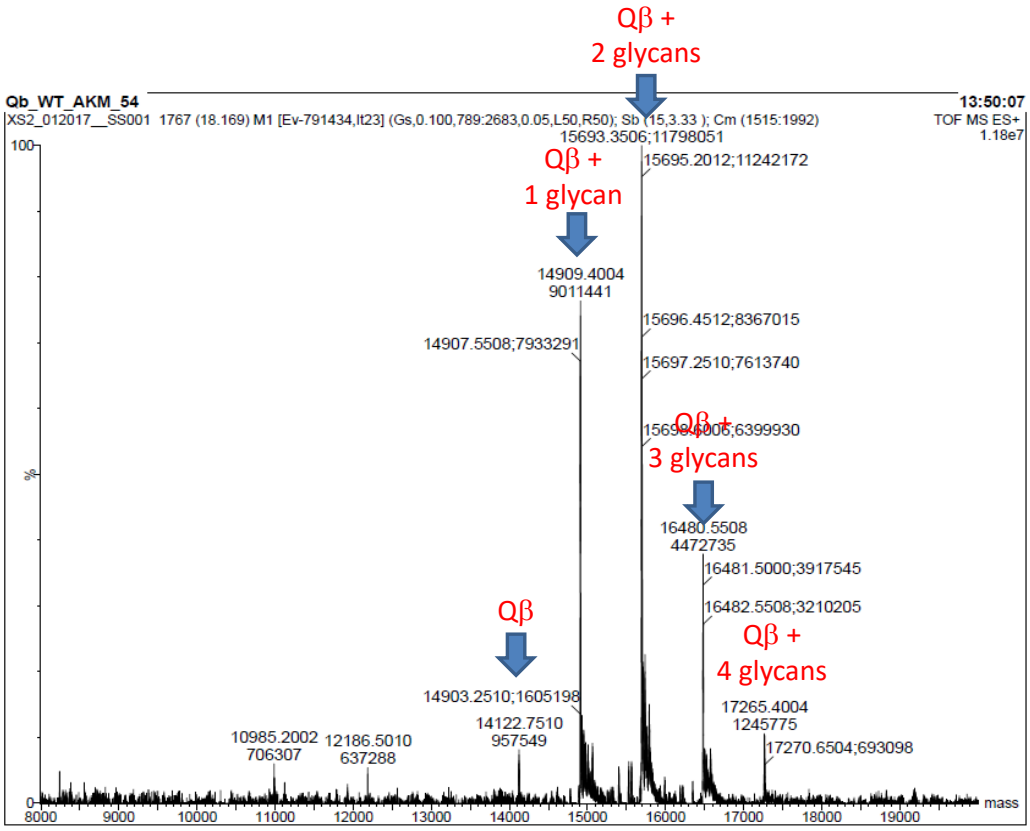
(300 mg, 0.20 mmol) in 0.1 M $CH_3ONa$ in $CH_3OH$ (10 ml) was stirred at room temperature for 2 h, neutralized with Dowex 50W X8 ($H^+$), filtered, and concentrated. A solution of the de-O-acetylated product in 80% AcOH (10 ml) was stirred at 80 °C for 2 h, concentrated and passed through a short pad of $SiO_2$. To a solution of the resulting mass in $CH_3OH$ (20 ml) was added 20% $Pd(OH)_2$-C (100 mg) and the mixture was stirred under a positive pressure of hydrogen for 16 h. The reaction mixture was filtered through a Celite® bed and the filtering bed was washed with $CH_3OH$-$H_2O$ (12 ml, v/v 1:1). The combined filtrate was concentrated under reduced pressure the crude product was passed through a Sephadex G-25 gel filtration column using water as the eluant to give pure compound **1** (75 mg, 57%). White powder; $[\alpha]_D^{25}$ +17 (*c* 1.0, $H_2O$); IR (KBr): 3415, 3058, 2828, 1325, 1275, 1096, 1021, 774, 698 $cm^{-1}$; $^1H$ NMR (500 MHz, $D_2O$): δ 5.04 (br s, 1 H, H-$1_C$), 5.00 (d, *J* = 3.0 Hz, 1 H, H-$1_A$), 4.99 (br s, 1 H, H-$1_B$), 4.88 (br s, 1 H, H-$1_D$), 4.09-4.08 (m, 3 H, H-$2_B$, H-$2_D$, H-$4_C$), 4.06-4.05 (m, 1 H, H-$2_A$), 4.02-3.93 (m, 4 H, H-$3_C$, H-$3_B$, H-$4_A$, H-$5_C$), 3.92-3.88 (m, 4 H, H-$2_C$, H-$3_A$, H-$5_B$, H-$6_{aC}$), 3.86-3.78 (m, 4 H, H-$5_A$, H-$5_D$, H-$6_{bC}$, OC*H*), 3.76-3.73 (m, 2 H, H-$6_{abA}$), 3.66-3.61 (m, 2 H, H-$4_D$, OC*H*), 3.59 (t, *J* = 9.5 Hz each, 1 H, H-$4_B$), 3.20-3.15 (m, 2 H, NC$H_2$), 2.05-2.03 (m, 3 H, H-$3_{aD}$, $CH_2CH_2CH_2$), 1.90-1.76 (m, 1 H, H-$3_{bD}$), 1.34 (d, *J* = 6.5 Hz, 3 H, C$CH_3$), 1.27 (d, *J* = 6.5 Hz, 3 H, C$CH_3$); $^{13}C$ NMR (125 MHz, $D_2O$): δ 101.9 (C-$1_C$), 101.3 (C-$1_B$), 101.2 (C-$1_D$), 98.4 (C-$1_A$), 81.4 (C-$4_B$), 78.0 (C-$2_C$), 77.3 (C-$3_A$), 73.3 (C-$3_C$), 71.0 (C-$2_B$), 70.3 (C-$2_D$), 70.1 (2 C, C-$3_B$, C-$5_B$), 69.0 (2 C, C-$2_A$, C-$4_A$), 68.0 (C-$5_A$), 67.1 (2 C, C-$4_C$, C-$5_C$), 66.8 (C-$5_D$), 65.7 (2 C, C-$4_D$, O$CH_2$), 61.1 (C-$6_A$), 60.5 (C-$6_C$), 37.6 (N$CH_2$), 33.3 (C-$3_D$), 26.5 ($CH_2CH_2CH_2$), 16.9, 16.7 (2 C, 2 C$CH_3$); HRMS calcd. for $C_{27}H_{49}NO_{18}$ (675.29): $[M+H]^+$ 676.3023; found: 676.3051. The structure of compound **1** was confirmed by NMR analysis [δ 5.04 (br s, 1 H, H-$1_C$), 5.00 (br s, 1 H, H-$1_A$), 4.99 (br s, 1 H, H-$1_B$), 4.88 (br s, 1 H, H-$1_D$) in the $^1H$ NMR and δ 101.9 ($J_{C1/H1}$ = 171.1 Hz, C-$1_C$), 101.3 ($J_{C1/H1}$ = 169.9 Hz, C-$1_B$), 101.2 ($J_{C1/H1}$ = 170.0 Hz, C-$1_D$), 98.4 ($J_{C1/H1}$ = 170.4 Hz, C-$1_A$) in the $^{13}C$ NMR spectrum].

**Synthesis and characterization of Qβ-glycan conjugate 1**

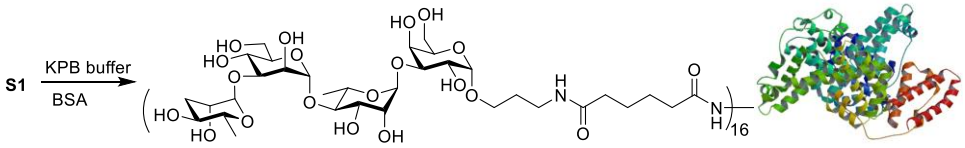



A solution of adipate bisNHS ester **16** (13 mg in 0.5 ml DMF, 0.037 mmol) was added into a solution of tetrasaccharide **1** (5 mg in 0.5 ml DMF, 0.007 mmol), which was followed by addition of di-isopropylethylamine (DIPEA, 1 μl, 0.007 mmol). The reaction mixture was stirred for 4 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by washing with DCM 3 times to give pure compound **S1** (5 mg, 75%). White solid; $^{1}$H NMR (500 MHz, $CD_3OD$): δ 8.03 (bs, 1H, NH), 5.05 (s, 1H), 4.91-4.89 (m, 1H), 4.83-4.82 (m, 2H), 4.04 – 3.66 (m, 18H), 3.58 – 3.46 (m, 3H), 3.40 (dt, *J* = 19.2, 6.2 Hz, 1H), 3.30 – 3.23 (m, 1H), 2.84 (s, 4H, $CH_2$ in NHS), 2.68 (dd, *J* = 8.5, 5.3 Hz, 2H, $COCH_2$), 2.25 (t, *J* = 6.8 Hz, 2H, $COCH_2$), 2.00 – 1.69 (m, 8H), 1.30 (d, *J* = 6.2 Hz, 3H), 1.22 (d, *J* = 6.0 Hz, 3H). To a solution of bacteriophage Qβ (5 mg, expressed and purified as previously described[4]) in potassium phosphate buffer (0.1 M, pH=7, 2.5 ml) was added a solution of **S1** (20 mg/ml in DMSO, 0.25 mL, 0.006 mmol). The reaction mixture was stirred overnight at room temperature on a tube rotator. The conjugate was purified by repeated filtration using an Amicon Ultra 100 kDa MW cut-off device against PBS buffer. Total protein content was quantified by Bradford assay against BSA standards. An average loading of 334 tetrasaccharide **1** per Qβ particle was determined by ESI-TOF LC-MS analysis (**Figure S1**).

**Figure S1.** ESI-TOF LC-MS of Qβ-glycan conjugate **1** with peaks from Qβ monomer and the respective Qβ–glycan conjugates marked. Assuming Qβ monomer and Qβ-glycan conjugates have the same ionizing efficiencies, the ion intensities of the peaks were used to estimate the relative quantities of each species. On average, there were 1.86 glycan 1 per Qβ monomer, which corresponds to 334 copies of glycan **1** per Qβ particle.




## Synthesis and characterization of BSA-glycan conjugate 1

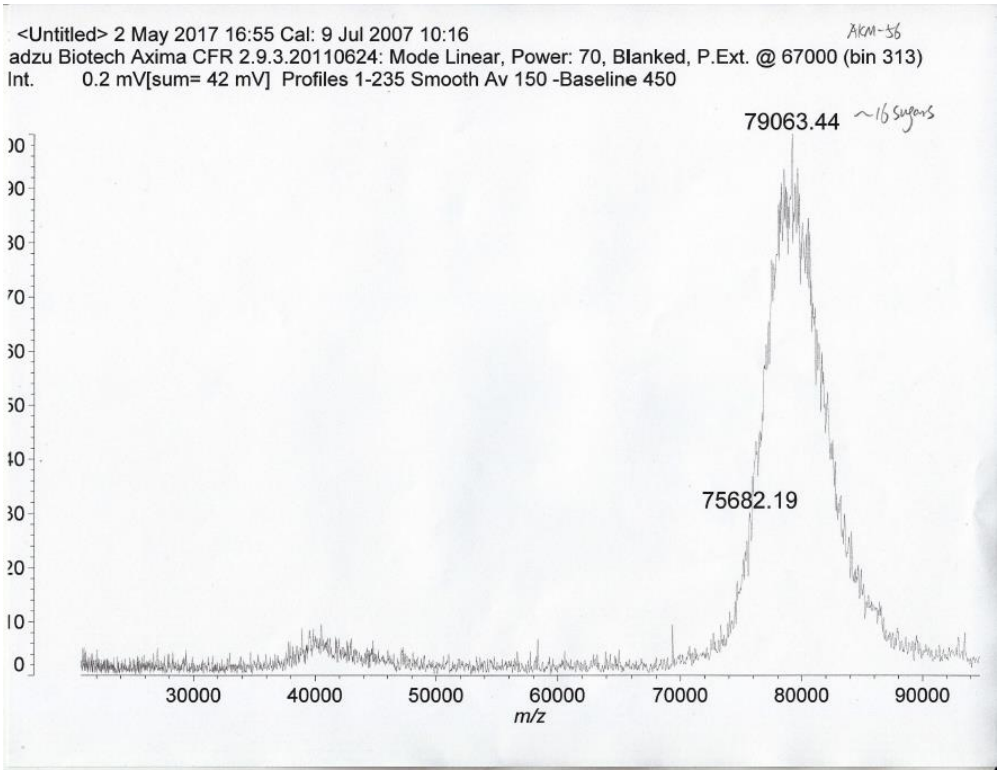



**BSA-glycan 1**

A solution of **S1** (20 mg/ml in DMSO, 0.1 ml, 0.002 mmol) was added to a solution of BSA (2 mg) in potassium phosphate buffer (0.1 M, pH=7, 1 ml). The reaction mixture was stirred overnight at room temperature on a tube rotator. The conjugate was purified by repeated filtration using an Amicon Ultra 10 kDa MW cut-off device against PBS buffer. Total protein content was

quantified by Bradford assay against BSA standards. A loading of 16 tetrasaccharides per BSA was determined using MALDI-TOF MS analysis (**Figure S2**).

**Figure S2.** MALDI-TOF MS of BSA-glycan **1** conjugate**.** Based on the molecular weight difference between the BSA-glycan **1** conjugate and unmodified BSA, the number of glycan 1 per BSA was calculated to be 16.



**Procedure for mouse immunization.**

Pathogen-free C57BL6 female mice aged 6-10 weeks were purchased from Charles River and maintained in the University Laboratory Animal Resources facility of Michigan State University. All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Michigan State University. C57BL6 mice were injected subcutaneously under the scruff on day 0 with 0.1 mL Qβ-glycan **1** construct (at 1 μg and 4 μg glycan respectively) as emulsions in Complete Freund's Adjuvant according to manufacturer's instructions. Boosters were given subcutaneously under the scruff on days 14 and 28 mixed with Incomplete Freund's Adjuvant. Sera samples were collected on days 0 (before immunization), 7 and 35.

**Procedure for rabbit immunization.**

Rabbit immunization studies were performed by ProSci Inc (Poway, CA). Two New Zealand rabbits were injected subcutaneously on day 0 with 0.1 mL Qβ-glycan **1** constructs (at 8 μg glycan) as emulsions in Complete Freund's Adjuvant according to manufacturer's instructions. Boosters were given subcutaneously on days 14, 28 and 42 mixed with Incomplete Freund's Adjuvant. Sera samples were collected on days 0 (before immunization), 35, 49 and 56.

**Evaluation of antibody titers and subtypes by ELISA.**

A 96-well Nunc microtiter plate was first coated with a solution of BSA-glycan conjugate or COPS (10 μg $mL^{-1}$, 100 μL/well) in $NaHCO_3/Na_2CO_3$ buffer (pH = 9.5) and incubated at 4 °C overnight. The plate was washed with PBST (4 x 200 μL), blocked with 1% BSA/PBS (200 μL/well) for 1 h at room temperature, washed with PBST (4 x 200 μL), and incubated with serial dilutions of anti-sera from immunized mice in 0.1% BSA/PBS (100 μL/well, 4 wells for each dilution). The plate was incubated for 2 h at 37 °C and then washed with PBST (4 x 200 μL). A 1:2000 dilution of HRP-conjugated goat anti-mouse IgG or goat anti-rabbit IgG (Jackson ImmunoResearch Laboratory) in 0.1% BSA/PBS (100 μL) was added to the wells respectively to determine the levels of antibodies generated. The plate was incubated for 1 h at 37 °C. A solution of enzymatic substrate was prepared by dissolving TMB (5 mg) in a mixture of DMSO (2 mL) and citric acid buffer (18 mL) in a 50 mL centrifuge tube covered with aluminum foil. $H_2O_2$ (20 μL) was added and the mixture was homogenized by vortexing. The plate was washed with PBST (4

x 200 µL) and a solution of enzymatic substrate was added (200 µL). Color was allowed to develop for 15 min and 0.5 M $H_2SO_4$ (50 µL) was added to quench the reaction. The absorbance was measured at 450 nm using a microplate reader. The titer was determined by regression analysis with log10 dilution plotted with optical density. The titer was reported as the highest fold of dilution (ELISA unit) that gives OD = 0.3.

**Bacterial strains and growth conditions**

*S. Enteritidis* R11 has been described previously[5] and was maintained in Hy-Soy media (Teknova, CA) as described.[6]

**Procedure for flow cytometry**

A single colony of *S. Enteritidis* R11 was grown in HS broth overnight at 37°C with shaking at 160 rpm. The next day, bacteria were adjusted to an $OD_{600}$ of 0.8 and placed on ice. 250 µL of bacteria were washed once with flow buffer (1% heat-inactivated FBS in PBS) and incubated with a 1:1,000 dilution of pooled rabbit sera (heat-inactivated for 30 min at 56°C) for 1 hr at 4°C. Rabbit sera tested included day 56 anti-sera from rabbits immunized with Qβ-glycan **1** or Qβ only, or the pre-immune sera. Bacteria were then washed two times with flow buffer, followed by incubation with 1 µg/mL of AlexaFluor 488-conjugated donkey anti-rabbit IgG (Thermo Fisher, IL) diluted in flow buffer for 1 hr at 4°C. Bacteria were then washed twice with PBS, fixed with 2% formaldehyde, and read using an LSR-II flow cytometer (BD Biosciences, NJ) with 2.5 x $10^5$ events recorded. As a negative control, bacteria were incubated with secondary antibody alone (red curve for unstained bacteria in **Figure 3**).

**Procedure for opsonophagocytosis assay**

Mouse macrophage J774 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 4.5 g/L D-glucose, L-glutamine, and sodium pyruvate (Corning, NY) supplemented with 10% v/v fetal bovine serum (Corning, NY) (referred to hereafter as D10) and 1% penicillin-streptomycin (Corning, NY). 24-well plates were seeded with 4.5 x $10^5$ J774 cells/well and incubated overnight at 37°C with 5% $CO_2$. The next day, confluent monolayers of J774 cells were washed three times with sterile PBS, and media was replaced with 500 µL of fresh antibiotic-free D10. Bacteria were prepared in the following manner: an overnight plate of *S.*

Enteritidis R11 (prepared from 3-5 colonies grown on solid Hy-Soy agar) was resuspended in PBS and adjusted to an $OD_{600}$ of 0.2. The culture was diluted 1:20 in PBS and incubated in either 10% pooled rabbit sera (heat-inactivated for 30 min at 56°C) or PBS as a negative control. Opsonization of R11 occurred for 20 min at room temperature with periodic gentle mixing. A suspension of opsonized bacteria (10 µL) was added to J774 cells at a Multiplicity of infection (MOI) of 0.1 – 0.4 (in 3-5 replicate wells). Afterwards, plates were swirled gently, centrifuged for 10 min at 1,000 rpm, followed by incubation for 50 min in a humidified 37°C incubator. The J774 cells were then washed once with PBS, and extracellular bacteria were killed by adding 500 µL/well of fresh D10 containing 100 µL/mL of gentamycin (Sigma, MO). After 1 hr incubation at 37°C/5% $CO_2$, J774 cells were washed three times with PBS, lysed with 500 µL of water purified from a Milli-Q Element Ultrapure Water System (EMD Millipore, MA), and placed on ice. Lysates were assessed for viable CFU after serial dilution in PBS. Results were expressed as fold uptake relative to the no sera control (i.e., the number of recoverable bacteria opsonized with pooled rabbit sera vs. PBS).

**Protection of mice from lethal challenges by *S*. Enteritidis**

All animal studies were performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and in compliance with guidelines for animal care established by the US Department of Agriculture Animal Welfare Act and Public Health Service policies. Animal experiments were conducted under protocols approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee (protocol #0715008). Pre-immune or day 56 post-vaccination rabbit sera ($n$ = 2/time point) were heat-inactivated at 56 °C for 30 min and diluted in PBS. Naïve 6-8-week old CD-1 female mice (Charles River Labs, MA) were injected intraperitoneally with the diluted pre- or post-immune rabbit sera (100 or 500 fold dilution, $n$ = 12/group) or PBS ($n$ = 7). After 3-4 hours, recipient mice were challenged intraperitoneally with 1 x $10^6$ CFU of *S.* Enteritidis R11. Infected mice were monitored daily, and animals that were moribund and/or had sustained ≥20% weight loss were euthanized and recorded as having succumbed to challenge. Vaccine efficacy for passively immunized mice was determined based on the proportionate attack rate (AR) in vaccinated and PBS control mice and was calculated as follows: $((AR_{control} - AR_{vaccinated})/AR_{control}) \times 100$.

**Figure S3. ELISA analysis for antibody subtypes**. The individual anti-sera (day 35 after 1st immunization) from mice immunized with Qβ-glycan **1** were assayed against BSA-glycan **1** conjugate. The antibody responses were mainly IgG type, with IgG2b, IgG2c and IgG3 as the main subtypes.

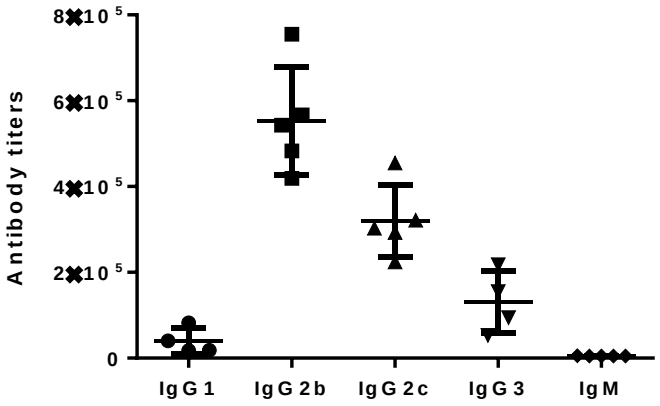



**Table S1.** ELISA titers of anti-glycan **1** IgG antibodies induced by Qβ-glycan **1** in rabbits. Robust anti-glycan **1** IgG antibodies were elicited compared to control rabbits receiving Qβ only.

| | **Day 35** | **Day 49** | **Day 56** |
|---|---|---|---|
| Rabbit #1 w/Qβ-glycan **1** | 46,000,000 | 80,000,000 | 83,000,000 |
| Rabbit #2 w/Qβ-glycan **1** | 53,000,000 | 183,000,000 | 150,000,000 |
| Control rabbit w/Qβ only | < 10,000 | < 10,000 | < 10,000 |

**Figure S4**. **ELISA analysis for antibody binding to native *S*. Enteritidis COPS**. Individual anti-sera from rabbits and mice immunized with Qβ-glycan **1** and various monoclonal antibodies were assessed for COPS binding by ELISA as indicated (all sera were diluted 25,000 times). X-axis labels: Pre-rabbit (pre-immunized sera from rabbits); Rabbit (day 35 sera from rabbits immunized with Qβ-glycan **1**); Mouse (day 35 sera from selected two mice immunized with Qβ-glycan **1**); MAb 6347 was against *S.* Paratyphi OPS; mAbs 6391 and 6393 were against the core polysaccharide of COPS.

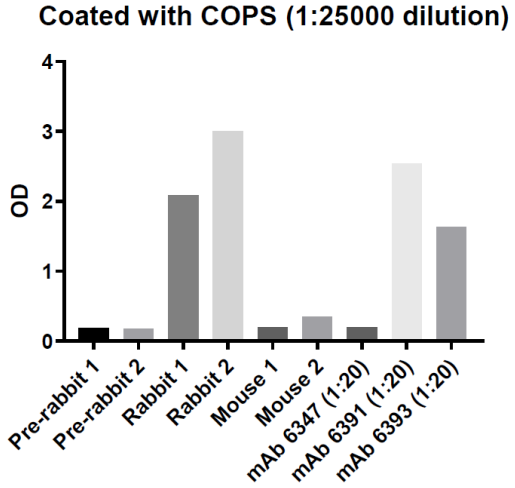
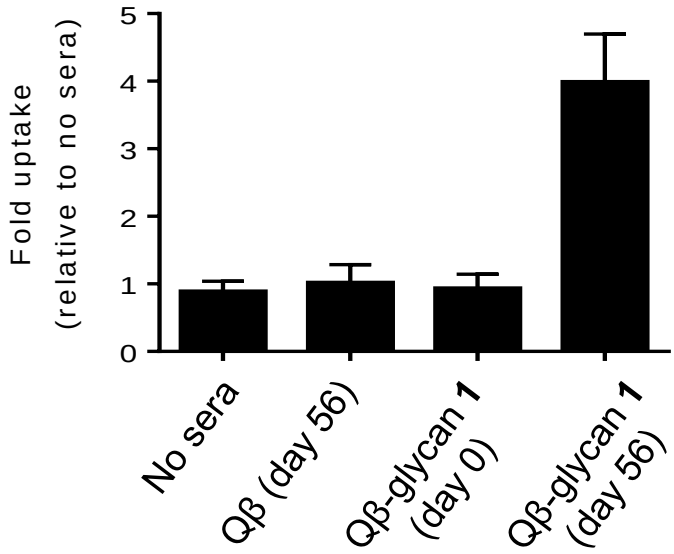



**Figure S5**. **Rabbit anti-Qβ-glycan 1 antibodies significantly enhanced OPA uptake into macrophage J774 cells.** *S.* Enteritidis R11 bacteria were incubated alone, with rabbit anti-Qβ sera, or with sera taken before, or after Qβ-glycan **1** immunization in rabbits. Mean and standard deviation from triplicate wells are shown. Results are representative of two independent experiments.



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