Supplemental Data for the Manuscript:

Nanomolar affinity anti-glycan antibody generation is controlled by T cells

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Supplemental Methods

Reagents. TS3, TS14 and PBS-57 were synthesized as described in the section "Compound synthesis" below. pCDF-1b and pET-11b plasmids with Qβ coat protein sequence (pET11b-CP and pCDF1b-CP) were constructed by Dr. S. D. Brown as described (1). Dulbecco's Phosphate Buffered Saline (DPBS) and chemically competent BL21(DE3) cells were purchased from Life Technologies (Carlsbad, CA). Acrylamide solutions and Coomassie Plus reagent were from Thermo Fisher, Waltham, MA. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO), unless noted otherwise.

Instrumentation. Continuous 10-40% sucrose gradients were prepared with a Biocomp Gradient Master and visualized with a Piston Gradient Fractionator (BioComp Instruments, Inc., Fredericton, NB, Canada). Size-exclusion and ion exchange chromatography analyses and purifications were performed on an Äkta Explorer (GE Healthcare, Piscataway, NJ). Microfluidic gel electrophoresis was performed on a 2100 Bioanalyzer using Series II Protein 80 chips (Agilent, Santa Clara, CA). All centrifugations were performed on Beckman Coulter centrifuges and rotors (Indianapolis, IN) at 4°C. MALDI-TOF spectra were collected on a Voyager DE Pro instrument (Applied Biosystems, Carlsbad, CA). Vacuum speed concentration was performed on a Savant SC110 Speedvac (Thermo Fisher, Waltham, MA). SPR measurements were taken on Biacore T200 (GE Healthcare, Piscataway, NJ). Confocal microscopy was carried out on Zeiss LSM 710 (Zeiss AG, Oberkochen, Germany).

Expression and purification of VLPs. BL21(DE3) *E. coli* cells were transformed with approximately 1 ng of a pET11b-CP or pCDF1b-CP plasmid. Expressions were carried out in 500 ml SOB media with IPTG induction at 37°C for 4-6 h. The cells were

then collected by centrifugation in a JLA-16.25 rotor at 10,000 rpm for 10 min, re-suspended in 0.1 M potassium phosphate buffer pH 7.0 and sonicated with a probe sonicator (10 min total sonication time, in cycles of 5 s on and 5 s off). The VLPs were precipitated from the resulting supernatant by the ammonium sulfate at 265 g/L (50% saturation) on ice overnight. The precipitate was resuspended in phosphate buffer and extracted with $CHCl_3$/nBuOH (1:1, v/v). VLPs were purified on 10–40% sucrose density gradients in an SW28 rotor at 27,000 rpm for 4-5 h. Visible particle bands were collected from each gradient and subsequently pelleted in an ultracentrifuge (50.2Ti rotor, 48,000 rpm, 2 h). The purified protein was dissolved in DPBS and filtered on a 0.2 μm filter. VLP purity and aggregation state were assessed by size exclusion chromatography and gel electrophoresis. Protein concentration was measured using Coomassie Plus reagent.

N-hydroxysuccinimide (NHS) acylation of VLPs. Qβ VLPs (5 – 30 mg, 0.35 – 2.1 μmol in CP) in 1xDPBS were mixed with water and 10x DPBS to give 2.77 mg/ml Qβ, 1.11x DPBS. NHS-alkyne linker was dissolved in DMSO at 17.54 mM (3.51 mg/ml) to make a 10x stock. The DMSO solution was then slowly added to the VLP solution for a final reaction of 2.5 mg/ml Qβ (175.4 μM in CP), 1.754 mM NHS-alkyne (10 eq. per CP) in 10% DMSO in 1x PBS. The entire mixture was reacted overnight at room temperature (RT). The following morning, the reaction mixtures were purified by either size-exclusion chromatography, or using repeated washes with 100 kDa MWCO filters (EMD Millipore, Billerica, MA).

Copper-catalyzed azide-alkyne cycloaddition reaction. For the synthesis of Qβ-TS14-40 conjugates, the final reaction conditions were as follows: 0.62 mg/ml Qβ-alkyne (43.5 μM in CP), 220 μM TS14-azide (~5 eq. per CP), 220 μM $CuSO_4$ (5 eq. per CP), 1.1

mM THPTA ligand (5 eq. per $CuSO_4$), 5 mM aminoguanidine, 5 mM sodium ascorbate, in 1xPBS. The particles and the glycan were first mixed together in buffer. $CuSO_4$ and THPTA were premixed to allow complex formation and added to the substrate mixture, followed by aminoguanidine. Sodium ascorbate was added last to initiate the reaction. The reaction proceeded for 4 hrs at RT and was purified by extensive washing with PBS using 100 kDa MWCO filters (EMD Millipore, Billerica, MA). To produce VLPs with different glycan loadings, the conditions were modified as follows: for Qβ-TS14-20: 73 μM TS14-azide (1.67 eq. per CP); for Qβ-TS14-80: 660 μM TS14-azide (15 eq. per CP); for Qβ-TS14-200: 1.25 mg/ml Qβ-alkyne (87.7 μM in CP), 2.11 mM TS14-azide (24 eq. per CP), 880 μM $CuSO_4$ (5 eq. per CP), 1.1 mM THPTA ligand (5 eq. per $CuSO_4$), 17.5 mM aminoguanidine, 17.5 mM sodium ascorbate, reaction proceeded overnight at RT.

MALDI analysis of Qβ CP. 10 μl of Qβ VLPs at 1 mg/ml were mixed with 10 μl of 1 M dithiothreitol (DTT) and 50 μl 10 M urea and incubated for 30' at 37ºC. 100 μl of 0.5 M iodoacetamide for 1 hr at 37°C in the dark. An additional 50 μL of 1 M DTT was added to the solution, which was left at RT for 10 min. Samples were dried using a vacuum speed concentrator, redissolved in 50 μl of 50% acetonitrile in water, and desalted using Cleanup C18 Pipette Tips (Agilent, Santa Clara, CA) according to the manufacturer's protocol. Samples were spotted using a sinapinic acid matrix.

Synthesis of BSA-TS14. Bovine Serum Albumin (BSA) was conjugated to TS14 bearing a carboxylic acid linker (TS14-COOH) using *in situ* NHS acylation, where TS14-COOH was first converted into an NHS ester. Equal amounts of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and NHS were combined together at RT in 10 mM MES buffer pH 4.5 to activate NHS. TS14-COOH was then added, for final

concentrations of 10 mM TS14-COOH, 20 mM NHS/EDC and 5 mM MES buffer, and NHS-ester of TS14-COOH was allowed to form for 5 minutes at RT. 5 mg/ml stock solution of BSA in PBS was then added directly to the reaction mixture for final concentrations of 3 mg/ml BSA, 4 mM TS14-NHS. The reaction was carried out overnight at RT, and BSA-TS14 was purified by size exclusion chromatography. Derivatization of BSA with TS14 was confirmed by MALDI mass spectrometry.

Synthesis of BSA-TS3 by strain-promoted azide-alkyne cycloaddition. BSA in PBS was mixed with 10x solution of monofluoro-substituted cyclooctyne (MFCO)-NHS (Berry & Associates, Dexter, MI) in DMSO for final concentrations of 2.5 mg/ml protein, 1.75 mM MFCO-NHS, and left to incubate overnight at RT. After purification by repeated washes with 30 kDa MWCO filters, BSA-MFCO (10 mg/ml) was incubated with TS3-azide (4.25 mM) in PBS at RT for 8 hours, then at 4ºC overnight, and purified with 30 kDa MWCO filters. Conjugation of BSA to TS3 was confirmed by MALDI mass spectrometry.

Human plasma isolation. 5 ml of blood from pediatric and adult donors was diluted 1:1 with PBS and overlayed on a layer of Ficoll-Paque (GE Healthcare, Piscataway, NJ). Density gradient centrifugation was carried out at 400 *g* for 25 minutes, with brakes off. Plasma was collected off the top of the liquid column.

ELISA. All manipulations were performed at room temperature unless stated otherwise, and all washes were performed with a volume of 150 µL per well. 96-well plates (Corning Inc., Corning, NY) were coated with 0.5 µg/mL BSA species in DPBS (100 µL), overnight at 4°C. The following morning, plates were washed 3x with 0.05% Tween 20 in DPBS (PBST) before blocking with 80 µl of 2% BSA in PBST (PBST-B) for 2 h. Dilutions of mouse sera (20 µl per well) were prepared in PBST-B, beginning with 1:10 to 1:20 for

titration experiments, or at 1:40 for set point dilution, unless specified otherwise, and added to blocked wells, for a final volume of 100 µl. After one hour, plates were washed three times with PBST, and 100 µL of a secondary donkey anti-mouse IgG or IgM horseradish peroxidase conjugated antibody (Jackson Immunoresearch, West Grove, PA) (1:5000 dilution in PBST-B) was added for 1 h. Plates were washed four times with PBST, and detection was accomplished with 100 µL of 0.4 mg/ml O-phenylenediamine dihydrochloride (OPD). Color was developed for 5-20 minutes at RT before quenching with 50 µL of 2 M H_2SO_4. Absorbance at 492 nm was recorded with a Sunrise microplate reader (Tecan, Männedorf, Switzerland).

***S. pneumoniae* propagation and staining.** *S. pneumoniae* serotype 14 (catalog number 6314) and serotype 3 (catalog number 6303) were obtained from ATCC. The bacteria were plated on blood agar plates (BD Biosciences, San Jose, CA) and grown overnight at 37°C in a 5% CO_2 atmosphere. Three milliliters of Brain-Heart Infusion (BHI) broth supplemented with a 1x1 cm brick of blood agar were inoculated with the individual colonies from the plate. Bacteria were grown to log phase (OD600 ~0.5) three times to obtain highly encapsulated strain (2), and stored at 4°C overnight between passages. Serial dilutions of bacterial cultures taken at different stages of growth were plated on blood agar plates to establish a linear correlation between OD600 and $\log_{10}$(CFU/ml). The formula derived for serotype 14 is: $\log_{10}(CFU_{14}) = 1.4xOD600 + 7.56$. For serotype 3: $\log_{10}(CFU_3) = 1.53xOD600 + 7.8$. For heat-inactivation bacterial suspensions were washed with PBS and incubated for 1 hr at 60°C.

Confocal microscopy. Heat-inactivated cultures of *S. pneumoniae* ($5x10^7$ CFUs before heat inactivation) were stained with sera from naïve and immunized mice at 1:100

dilution for 30’ at RT, followed by staining with Cy3-conjugated donkey anti-mouse IgG (Jackson Immunoresearch) for 30’ at RT, and incubation with 1:1000 Hoechst 33342 (Thermo Fisher) for 5’. Bacterial cell suspensions were plated on glass slides in antifade reagent (Thermo Fisher) and imaged using Zeiss LSM 710 confocal microscope. Images were analyzed using the Zen software (Zeiss AG, Oberkochen, Germany).

Transmission electron microscopy. $5x10^6$ CFU of heat-inactivated *S. pneumoniae* serotype 14 were washed with HBSS buffer, incubated in HBSS, 4% normal goat serum and 14.22 at 10 µg/ml for 1 hr at 4°C, washed 3 times with HBSS, incubated with HBSS, 4% normal goat serum and 12 nm gold-labeled goat anti-mouse IgG (immunogold) for 2 hrs at 4°C, and washed 3 times with HBSS again. All washes were performed in an Eppendorf microcentrifuge at maximal speed (16100g rcf) for 5’, except the last washes (after immunogold incubation), which were performed at 9300g rcf to prevent non-specific immunogold sedimentation. The suspension of immunogold labeled cells were first fixed in ice cold 2.5% glutaraldehyde in 0.1M cacodylate buffer, and after a brief wash, pelleted and fixed in 1% osmium tetroxide. The pellets were dehydrated in graded ethanol series, treated in propylene oxide and embedded in EMbed 812/Araldite (Electron Microscopy Sciences, Hatfield, PA). The pellets were then re-embedded for subsequent sectioning to provide transverse profiles of the pellets. Thick sections (2µm) were cut, mounted on glass slides and stained in toluidine blue for general assessment in the light microscope. Subsequently, 70nm thin sections were mounted on parlodion-coated copper slot grids and stained with uranyl acetate and lead citrate for examination at 80kV on a Philips CM100 electron microscope (FEI, Hillsbrough OR). Images in tif format were documented using a Megaview III ccd camera (Olympus Soft Imaging Solutions GmbH,

Münster, Germany) and subsequently handled in GIMP.

ELISPOT. On the night before the experiment, dilutions of BSA (2 µg/ml and 5 µg/ml) and BSA-TS14 (1 µg/ml, 2 µg/ml and 5 µg/ml) in 100 µl were added to the wells of the ELISPOT plate (BD Biosciences, San Jose, CA), and the plate incubated at 4ºC overnight. 5 days after intravenous immunization with Qβ-TS14 conjugates mouse spleens were harvested, and single cell suspensions were generated by passing the cells through a 70 µM cell strainer (Corning Inc., Corning, NY) in sterile conditions. The ELISPOT plate was washed 5x with sterile PBS and blocked with 200 µl/well RPMI with 10%FBS for 30' at RT. Suspensions of $5x10^5$, $2.5x10^5$ and 10^5 splenocytes in 100 µl were added to the wells of the ELISPOT plate in triplicates and incubated at 37ºC, 5% CO_2 in the dark for 18 hours. The plate was washed 5x with PBS to remove cells and incubated with biotinylated anti-mouse IgG (1µg/ml in PBS with 5% FCS) for 2 hours at RT. After five washes with PBS, 100 µl/well of TMB substrate solution (Thermo Fisher, Waltham, MA) were added and incubated until colored spots developed. The plate was washed with tap water, dried and stored at RT in the dark. Spots were counted using a QuantiHub reader (MVS Pacific, Roseville, MN).

Production of B-cell hybridomas. Previously immunized C57BL/6 mice were boosted with the same Qβ formulation, followed by a final i.v. boost two weeks later without the adjuvant. On the same day, P3-x63-Ag8.653 mouse myeloma cells (ATCC CRL-1580) were recovered from cryopreservation and expanded to exponential cultures. Three days after the boost, spleens were harvested and splenocytes fused at a 4:1 ratio with mouse myeloma cells, using 50% (w/v) Hybri-Max polyethylene glycol (PEG) solution (Sigma-Aldrich, St. Louis, MO). Fused cells were selected in media containing

hypoxanthine-aminopterin-thymidine (Sigma-Aldrich, St. Louis, MO) for 10 days, followed by an ELISA screen against BSA-TS14 or BSA-TS3. Positive clones were expanded in complete RPMI 1640 media containing hypoxanthine-thymidine (Sigma-Aldrich, St. Louis, MO). Positive wells were subcloned and retested by ELISA. All hybridoma cell lines were isotyped using an ELISA-based assay using isotype-specific antibodies (Jackson ImmunoResearch, West Grove, PA).

RNA isolation and RLM-RACE. Hybridomas were grown to approximately $5x10^6$ cells, and total RNA extracted using TRIzol (Life Technologies, Carlsbad, CA). FirstChoice RLM-RACE Kit (Life Technologies, Carlsbad, CA) was used for cDNA synthesis and amplification. According to the manufacturer's protocol, a 45 base RNA adapter oligonucleotide was ligated to the 5' end of full length mRNA, followed by reverse transcription with M-MLV reverse transcriptase and random decamers. Variable regions of both heavy (V_H) and light (V_L) chains were amplified using a 5' primer complementary to the adapter sequence, and 3' primers complementary to either the constant region of the kappa light chain or the first domain of the constant region (C_H1) for each heavy chain respectively, as described (3). 5 µl of cDNA were used for each PCR in a reaction volume of 50 µl with the final concentrations of 0.2 mM dNTP (Roche, Indianapolis, IN), 1.5 mM $MgCl_2$ (Roche, Indianapolis, IN), 0.5 µM Betaine (Sigma-Aldrich St. Louis, MO), 2.5 U of Taq DNA polymerase (Roche, Indianapolis, IN) and 0.4 µM of each primer. The thermal cycling profile was as follows: initial melting at 95ºC for 3 minutes, 30 cycles of 95ºC for 15 seconds, 40ºC (V_H) or 50ºC (V_L) for 30 seconds, and 72ºC for 1.30 minutes, with a final elongation at 72ºC for 30 minutes.

TA cloning. PCR products were separated on a 1% agarose gel and DNA of the

expected size extracted using Geneclean III Kit (MP Biomedicals, Santa Ana, CA). 2 µl purified DNA was cloned into the pCR 2.1-TOPO vector (Life Technologies, Carlsbad, CA) and transformed into DH5α competent cells. After overnight incubation on kanamycin plates with X-Gal, white colonies were grown in LB with carbenicillin and minipreps performed for plasmid isolation. Clones containing inserts of the expected size were determined by restriction enzyme digestion with EcoR1 (Roche, Indianapolis, IN).

DNA sequencing and analysis. Samples were sequenced by Sanger DNA sequencing (GENEWIZ, Inc.) using the T7 promoter primer. The sequences were aligned and compared to the mouse immunoglobulin database of IMGT (4).

In-solution competition experiments. Fab14.22 at concentrations ranging from 62.5 nM to 2000 nM was injected onto BSA-TS14-derivatized surface of the CM5 chip to create a calibration curve. 500 nM Fab14.22 was incubated with increasing concentrations of free TS14, and these mixtures were injected onto the same surface. The calibration curve was then used to obtain the calculated concentration of Fab14.22 at each inhibitor concentration. These calculated concentrations were plotted against TS14 concentrations, and the inhibition curve was fit using Biacore T200 Evaluation software to obtain the dissociation constant.

Papain digestion of antibodies to produce Fab fragments. IgG antibodies were washed with 100 mM NaOAc pH 5.5 and 1 mM EDTA. 300 to 1500 ng papain (per 1 mg antibody) were pre-activated in 100 mM NaOAc pH 5.5, 1 mM EDTA, 50 mM cysteine for 15 min at RT, and the antibody solution added to 1 mg/ml. Reaction was carried out at 35°C for 30 min to 2 hours with occasional agitation. Reaction was quenched by 70 mM iodoacetamide, and the Fab isolated from Fc and uncleaved antibody by a Protein A

column (GE Healthcare, Piscataway, NJ). The purity of the Fabs was confirmed by PAGE gel. The Fabs were then purified on a Superdex 75 size-exclusion column with PBS as a mobile phase.

Crystal structures of Fab14.22 and Fab14.22-TS14 complex. Crystals of Fab14.22 (5.6 mg/ml) were formed in 1:1 (v/v) protein/reservoir drop equilibrated against 3.6M ammonium sulfate, with 10% PEG400 and 10%MPD, in 1M of HEPES (pH 7.5) reservoir solution. Crystals for the complex between the tetrasaccharide and Fab14.22 (10:1 ligand:protein) were obtained in 0.8 M NaHPO4/1.2 M K_2HPO4, 0.1M sodium acetate (pH 4.5), and 5% Jeffamine 900.

Data collection and crystal structure determination. Data for unliganded Fab14.22 and its complex with TS14 were collected at the Advanced Photon Source (APS) of the Argonne National Laboratory at beamlines 23 ID-D and 23ID-B, respectively. Data were indexed and processed using HKL2000 (5). The Fab14.22 crystal structure (PDB ID: 5JOR) was solved by molecular replacement using the coordinates from PDB ID 1QGC as the search model, while the coordinates from the apo-form of Fab14.22 were used as a search model for the tetrasaccharide-Fab14.22 complex (PDB ID: 5JOP) with Phaser (6). Structure refinement was carried out with Phenix (7) and modeling with Coot (8). Data quality and refinement statistics are outlined in Table S5. Figures were generated using PyMOL (9) and LigPlot (10). The buried surface area of the Fab14.22 complex was calculated using MS (11).

Peptide and glycopeptide synthesis.

Peptides. Sequences of the peptides used are provided in Supplemental Table 6. p13-alkyne and p16-alkyne precursor peptides were purchased from Avanti Polar

Lipids. Qβ peptides for T cell restimulation and intracellular cytokine staining were selected in Qβ coat protein sequence based on the Immunoepitope database (IEDB) prediction of best binders of MHC class II I-Ab (12). Each Qβ peptide pool was formed by one of the three peptides from different regions of Qβ coat protein with the highest ranking in the binding prediction algorithm, and two neighboring peptides offset by one amino acid (see Supplemental Table 6). Peptides were synthesized at the La Jolla Institute for Allergy and Immunology using standard Fmoc chemistry. Extended 13-mers p13*-alkyne and p16*-alkyne were chain assembled by manual Fmoc-SPPS, using 0.1 mmol pre-loaded resin. (Fmoc-Glu-Wang, 0.55 mmol/g; Fmoc-Leu-Wang, 0.75 mmol/g; Fmoc-Asn-PEG-HMPA, 0.75 mmol/g). During chain assembly, Fmoc protecting groups were removed by treating the resin with 2 washes of a solution of 20% 4-Methylpiperidine in DMF for 90 s. Except where noted, for coupling, Fmoc-amino acids (0.5 mmol) were dissolved in 1.25 mL of 0.4 M HCTU in DMF (0.5 mmol), and DIEA (0.75 mmol, 130 µL) was added. After 30 s, the solution was added to the resin. Coupling times were 25 min. Alternatively, Fmoc-Lys(ivDde)-OH (0.2 mmol, 115 mg) was dissolved in 0.5 mL of 0.4 M HATU in DMF (0.2 mmol) and DIEA (0.3 mmol, 38.7 µL) was added. After 30 s, the solution was added to the resin. Following chain assembly, the terminal Fmoc group was removed and Boc-anhydride (0.25 mmol, 54.5 mg) was dissolved in 0.5 mL of DMF and DIEA (0.5 mmol, 43.5 µL) was added. After 30 s, the solution was added to the resin. Coupling time was 30 min, and coupling efficiency was checked with a Kaiser test. The Lys side chain protecting group ivDde was removed with 4 washes of 4% hydrazine hydrate in DMF for 5 min. After deprotection, the resin (0.05 mmol) was treated to incorporate 4-Pentynoic acid. 4-

Pentynoic acid (0.1 mmol, 9.8 mg) was dissolved in 250 µL of 0.5 M HCTU in DMF (0.1 mmol) and DIEA (0.15 mmol, 26.1 µL) was added. After 30 s, the solution was added to the resin. Coupling time was 30 min, and coupling efficiency was checked with a Kaiser test. Peptide p16*-alkyne was synthesized using the building block Fmoc-Asp(OtBu)(Dmb)GlyOH to preclude aspartimide formation. Peptides were cleaved from the resin using a cleavage cocktail that contained TFA (95%), TIS (2.5%) and H2O (2.5%). Resins were treated with the cleavage cocktail for 120 min. Afterwards the resin was filtered and TFA was evaporated using a gentle stream of N2 over the mixture. The crude peptides were precipitated with cold ether, and dissolved in 30% Buffer B (0.05% TFA, 90% CH3CN, 10% H2O) in Buffer A (0.05% TFA in H2O) and lyophilized.

gp13 and gp16*.* The following solutions were made: 20 mM p13-alkyne in water, 19 mM p16-alkyne in DMSO/water (1:4), 20 mM azido sugar TS14 in DMSO, 20 mM CuSO4 in water, 20 mM tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) ligand in water, 20 mM aminoguanidine in water, 20 mM sodium ascorbate (made before use). Reagents were added in the following sequence: 200 µL p13-alkyne or 260 µL p16-alkyne (4 µmol*,*1eq), TS14 (1.2 eq, 4.8 µmol, 240 µL), premixed CuSO4 (2eq, 8 µmol, 400 µL)/ligand (2 eq, 8 µmol, 400 µL) solution, aminoguanidine (5 eq, 20 µmol, 1000 µL), sodium ascorbate (5 eq, 20 µmol, 1000 µL). Reaction mixture was stirred gently at room temperature overnight. The product was isolated using a semi-preparative HPLC Restek C18 column (#9604577) with gradient 0-20% CH3CN/0.1% TFA over 50 min for gp13 and 0-40% CH3CN/0.1% TFA over 50 min for gp16. Fractions were lyophilized to give 9.2 mg of gp13 (pale solid; MS, m/z 994 (MH^{2+})) and 9.0 mg of gp16 (white solid; MS, m/z 1015 (MH^{2+})).

gp13*. A solution of GP13 (2.51 mg, 1.8 μmol) in DMSO (123 μL), was added TS14 (43 μL from a 100 mM solution in H2O, 2.16 μmol), THPTA (108 μL from a 50 mM solution in H2O, 5.4 μmol), CuSO4.5H2O (21.6 μL from a 50 mM solution in H2O, 1.08 μmol), amino guanidine (108 μL from a 100 mM solution in H2O, 10.8 μmol), and freshly prepared sodium ascorbate (108 μL from a 100 mM solution in H2O, 10.8 μmol). The solution was stirred at 37 °C for 10 hrs. The solution went from colorless to pale yellow. Upon consumption of TS14 as monitored by ESI-MS, the reaction was diluted to 1 mL with H2O and the crude was purified by semi-preparative RP-HPLC (column = Zorbax SB-C18 (5 μm, 9.4x250mm); linear gradient of 10 to 50% MeCN+0.1%TFA/H2O+0.1%TFA during 20 min; flow rate=5.0 mL/min). Fractions containing the product were lyophilized to dryness. Mass of product = 1.4 mg and mass of recovered GP13 = 1.4 mg. See Supplemental Figure 10 for ESI-MS data.

gp16*. A solution of GP16 (3.12 mg, 2.1 μmol) in DMSO (113.4 μL), was added TS14 (50 μL from a 100 mM solution in H_2O, 2.52 μmol), THPTA (126 μL from a 50 mM solution in H_2O, 6.3 μmol), $CuSO_4{\cdot}5H_2O$ (25.2 μL from a 50 mM solution in H_2O, 1.26 μmol), amino guanidine (126 μL from a 100 mM solution in H_2O, 12.6 μmol), and freshly prepared sodium ascorbate (126 μL from a 100 mM solution in H_2O, 12.6 μmol). The solution was stirred at 37 °C for 10 hrs. The solution changed from colorless to pale yellow. Once the TS14 was consumed as monitored by ESI-MS, the reaction was diluted to 1 mL with H_2O and the crude was purified by semi-preparative RP-HPLC (column = Zorbax SB-C18 (5 μm, 9.4x250mm); linear gradient of 10 to 50% MeCN+0.1%TFA/H_2O+0.1%TFA during 20 min; flow rate=5.0 mL/min). Fractions containing the product were lyophilized to dryness. Mass of product = 1.4 mg and mass of recovered GP16 = 1.7 mg. See Supplemental

Figure 10 for ESI-MS data.

I-A^b purification, loading and western blotting. I-A^b-CLIP with a thrombin cleavage site to remove the CLIP peptide was expressed and purified as described (13, 14). The protein was cleaved by thrombin (5 units per 1 mg I-A^b) at 37ºC for 1 hour and then overnight at RT. Thrombin was inactivated by adding 1mM Pefabloc, and cleaved I-A^b was purified by size exclusion chromatography. Glycopeptides (20- to 200-fold excess over I-A^b) were loaded onto I-A^b in 200 mM malonate pH 5.0 in the presence of recombinant HLA-DM (1:10 to I-A^b) for 1-3 days at RT. For western blotting, the complexes were separated by native gel electrophoresis, transferred to PVDF membranes, and blots developed using LI-COR Odyssey infrared imaging system (Li-COR, Lincoln, NE). For flow cytometry, the I-A^b-glycopeptide complexes were incubated with PE-labeled streptavidin (SNN1007, Thermo Fisher, Waltham, MA) at 4:1 molar ratio in PBS for 3 hours to overnight to form streptavidin tetramers, and used in staining without further purification.

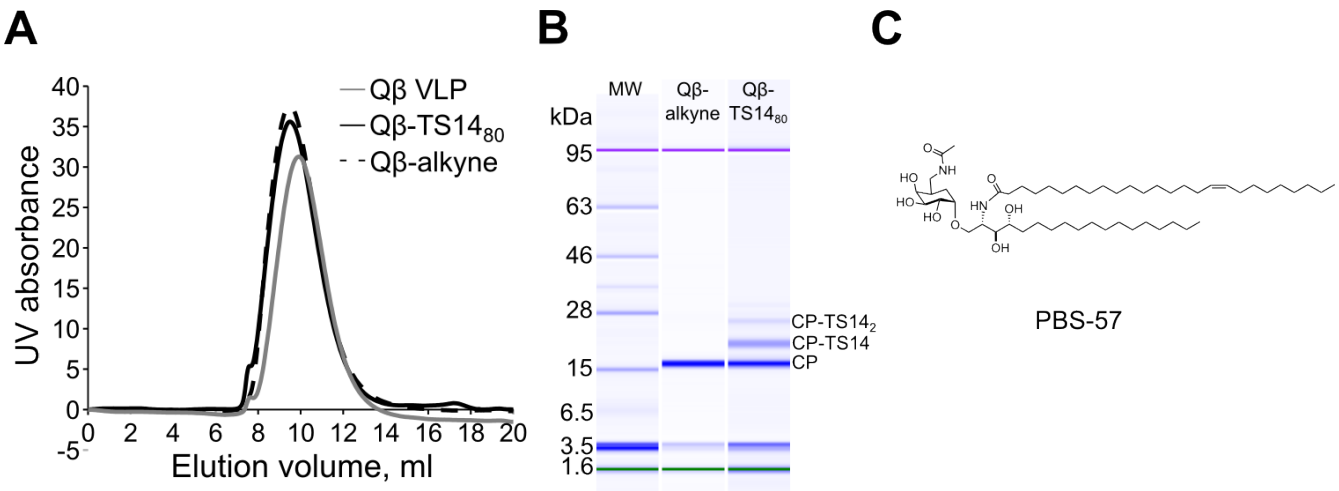
Flow cytometry. 5 days after intravenous or intramuscular immunization mouse splenocytes, or inguinal, popliteal and periaortal lymph nodes, respectively, were harvested, and single cell suspensions were generated by passing the cells through a 70 μM cell strainer. All incubations were carried out in PBS with 2% fetal bovine serum and 2 mM EDTA. Red blood cells were lysed in 0.165 M NH_4Cl solution at RT for 5', and the cells were incubated in Fc block for 15 minutes at 4ºC. 250 nM streptavidin-I-A^b tetramers were then added for 1 hour at RT. After washing, cells were incubated with directly conjugated anti-CD3ε-FITC (clone145-2C11), anti-B220-APC (clone RA3-6B2), anti-CD8α-APC (clone 53-6.7), anti-CD49b-APC (clone DX5), anti-CD11b-APC (clone

M1/70), anti-CD4-APC-Cy7 (clone RMA-5), all from BioLegend (San Diego, CA), for 15 minutes at 4ºC. Flow cytometry was carried out on Miltenyi MACSQuant (Miltenyi Biotec, Bergisch Gladbach, Germany) with propidium iodide added to enrich for live cells. Gating and population analysis was done using FlowJo (FlowJo LLC, Ashland, OR).

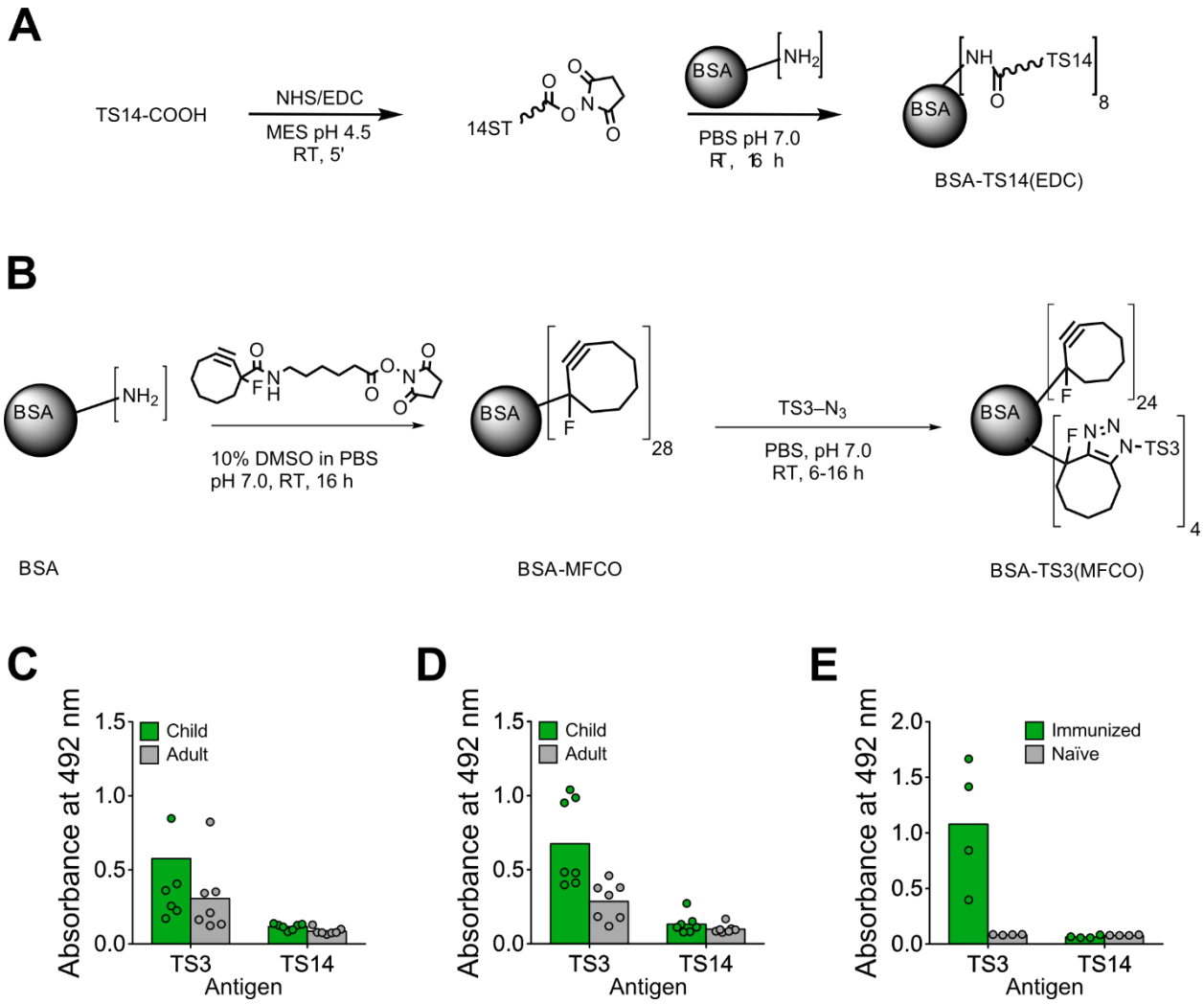
Intracellular cytokine staining. 5 days after secondary intramuscular immunization mouse inguinal, popliteal and periaortal draining lymph nodes were harvested and homogenized in complete RPMI with 10% FCS, 2 mg/ml Collagenase D (Roche, Indianapolis, IN) and 100 µg/ml DNAse I (Sigma-Aldrich, St. Louis, MO) for 1 hour at 37ºC in 5% CO_2. The reaction -was stopped by adding EDTA to 10 mM. Cells were counted, and $5x10^5$ to $1x10^6$ cells per sample were incubated in complete RPMI with 10% FCS with 10 µg/ml Qβ peptide or glycopeptide pools in the presence of 2 µg/ml anti-CD28 (clone 37.51, from BD Biosciences, San Jose, CA) for 5 hours. Brefeldin A (Sigma-Aldrich, St. Louis, MO) was added to 10 µg/ml after the first hour. After wash cells were incubated in FACS buffer (PBS with 2% fetal bovine serum and 2 mM EDTA) with 10 µg/ml 2.4G2 Fc block and directly conjugated anti-CD3ε-BV510 (clone 145-2C11), anti-B220-PE-Cy7 (clone RA3-6B2), anti-CD8α-PE-Cy7 (clone 53-6.7), anti-CD11b-PE-Cy7 (clone M1/70), anti-CD4-APC-Cy7 (clone RMA-5) and anti-CD44-Pacific Blue (clone IM7), all from BioLegend (San Diego, CA), for 20 minutes at 4ºC. Cells were then fixed and permeabilized using BD Biosciences Cytofix/Cytoperm kit, according to manufacturer instructions. Cells were left overnight in Perm/Wash solution at 4ºC. Next morning, cells were stained in Perm/Wash solution with directly conjugated anti-IFNγ-APC (clone XMG1.2) and ant-TNFα-FITC (clone MP6-XT22), from BioLegend (San Diego, CA), for 20 minutes at 4ºC. Flow cytometry was carried out on Miltenyi MACSQuant (Miltenyi

Biotec, Bergisch Gladbach, Germany). Gating and population analysis was done using FlowJo (FlowJo LLC, Ashland, OR).

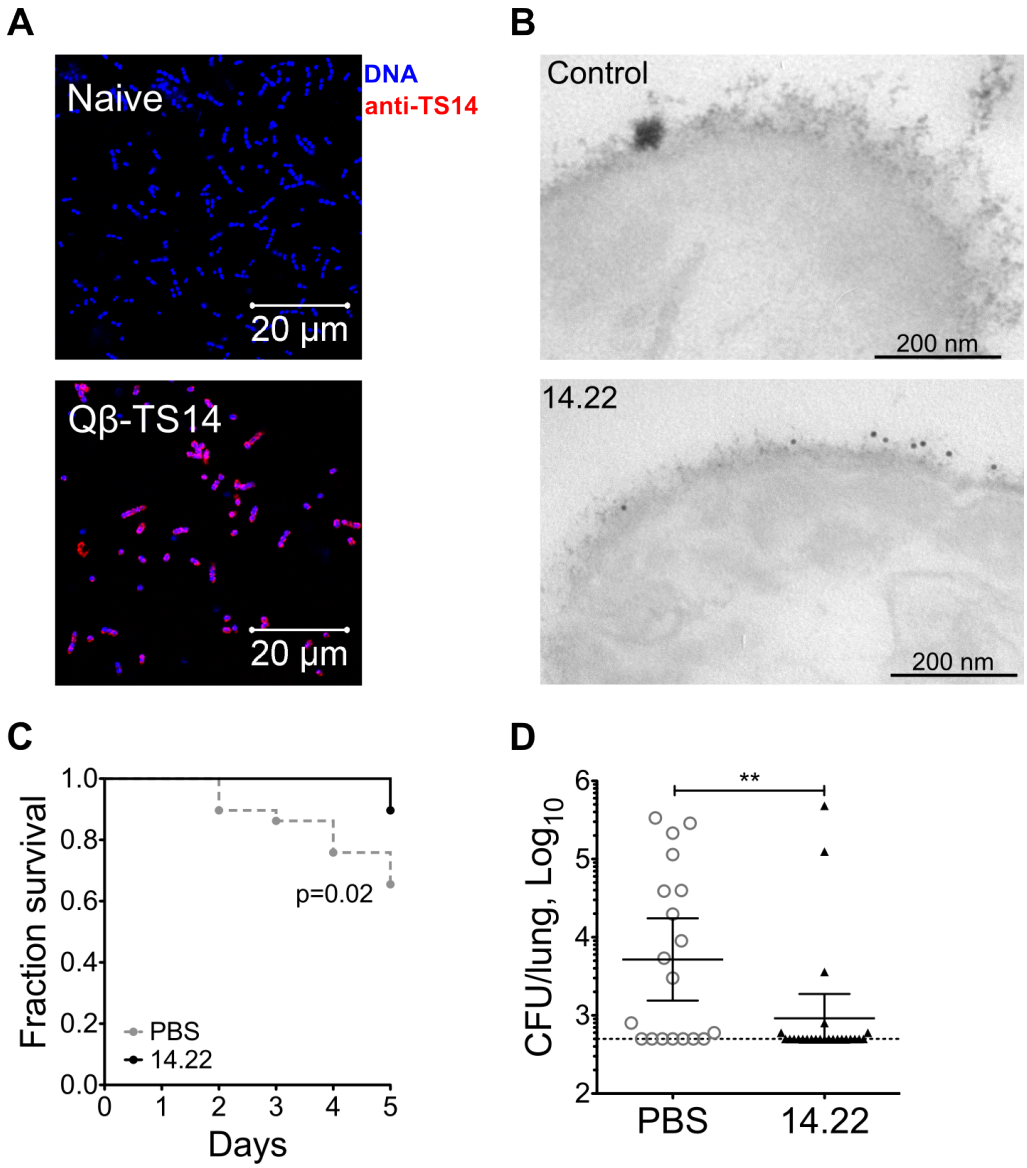
Supplemental Figures



Supplemental Figure 1. VLP characterization and molecules used in the study. (**A**) Gel filtration chromatograms of Qβ VLP, Qβ-alkyne and Qβ-TS14-80 on Superose 6 column. (**B**) Representative chromatograms of Qβ-alkyne and Qβ-TS14-80 obtained by microfluidic gel electrophoresis; conjugation of the sugar causes a shift in the electrophoretic mobility of the protein subunit, resulting in a separate peak on the chromatogram. (**C**) Chemical structure of the adjuvant PBS-57.



Supplemental Figure 2. BSA-TS antigens for ELISA. (**A**, **B**) Synthesis of test antigens for ELISA and SPR. For both TS14 and TS3 the carrier protein and the chemical linkage are changed to avoid detecting antibodies against Qβ or the linker. (**A**) Synthesis of BSA-TS14. (**B**) Synthesis of BSA-TS3. (**C-E**) Response of Prevnar-immunized humans and mice to short synthetic glycans. (**C**) Human plasma IgG response to BSA-TS3 and BSA-TS14 at 1:200 dilution. (**D**) Human plasma IgM response to BSA-TS3 and BSA-TS14 at 1:200 dilution. (**E**) Mouse serum IgG response to BSA-TS3 and BSA-TS14 at 1:100 dilution.



Supplemental Figure 3. Anti-TS14 antibodies bind *S. pneumoniae* serotype 14 capsule and are protective against infection in a passive immunization model. (**A**) Fluorescent images of *S. pneumoniae* serotype 14 stained with naïve or Qβ-TS14-immunized mouse sera. (**B**) Transmission electron microscopy images of *S. pneumoniae* serotype 14 stained with 14.22 antibody and a gold nanoparticle-conjugated secondary antibody, or secondary antibody alone. (**C**) Survival of NOD/SCID mice after intra-tracheal infection with 10^7 - 10^8 CFUs of *S. pneumoniae* serotype 14. The animals were injected intra-peritoneally with either PBS or 100 µg IgG14.22 24 hours prior to infection. 29 mice per group, pooled from 6 independent experiments. (**D**) Titers of *S. pneumoniae* in the lungs of infected mice 5 days after infection. Dashed line: limit of detection.

A

Heavy chain

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
14.2	EVQLQQSGPELVRPGSSVKMSCKTS	GFSFITYA	INWLKQRPGQGLEWIGY	VYIGNGYT	DHNKKFKDKATLTSDPSSSTAFMQLSSLTSEDSGIYFC	ARRGYPWSFDF	WGTGTTVTVSS
14.6	QVQLQQPGAELVKPGASVKLSCKAS	GYTFTSYW	MHWVKQRPGQGLEWIGM	IHPNSGSS	KHNEKFKSKATLTVDKSSNTAYMQLSSLTSEDSAVYYC	ARSDFYGNWYFDV	WGTGTTVTVSS
14.10	EVQLQQSGTELVRPGSSVKMSCKTS	RYTLTTHA	INWVKQRPGQGLEWIGY	IYIGNGYS	DYNEKFKGKATLTSDTSSSTAYMQLSSLTSTDSAIYFC	TRRGYPWYFDVW	WGTGTTVTVSS
14.13	EVQLQQSGPELVKPGASVKMSCKAS	GYTFTDYY	IHWVKQSHGKSLEWIGY	IYPFNGVT	TYNQNFKGKATLTVNMSSSTAYMELRSLTSDDSAVYYC	ARWDS	WGQGTTLTVSS
14.15	EVQLQQSGAELVRPGSSVKMSCKTS	GFSITKYA	INWLKQRPGQGLEWIGY	IYIGNGYT	DYNEKFTGKATLTSDTSSKTAYMHLSSLTSEDSALYFC	ARRGYPWYFDV	WGTGTTVTVSS
14.17	EVQLQQSGPELVKPGASVKMSCRAS	GYTFTEYY	IHWVRQSHGKSLEWIGY	VHPNDGGT	TYNQKFRGKATLTVNRSSDTAYLELRSLTSEDSAVYYC	ARWDY	WGQGTTLSVSS
14.18	EVQLQQSGPELVKPGASVKMSCEAS	GYTFTEYY	IHWVKQSHGKSLEWIGY	IHPNTGDA	TYNQNFRGKATLTVSRSSNTAYMELRSLTSEDSAVYYC	ARWDS	WGQGTTLTVSS
14.20	EVQLQQSGPELVKPGASVKMSCEAS	GYTFTEYY	IHWVKQSHGKSLEWIGY	IHPNTGDA	TYKQNFRGKATLTVSRSSNTAYMELRSLTSEDSGVYYC	ARWDS	WGQGTTLTVST
14.21	QVQLQQPGAEVVTPGASVKLSCKAS	GYVFTIYY	IHWVKQRPGQGLDWIGM	IHPNTGNT	NYNEKFRSKATLTVDRSSNTAYMQLSSLTSEDSAVYYC	ARWDY	WGQGTTLTVSS
14.22	EVQLQQSGPELIKPGASVKMSCEAS	GYIFTEYY	IHWVKQIQGRSLEWIGY	VHPKTGDV	IYNQNFRGKATLTVNRSSNTAYMELHSLTSEDSAVYYC	ARWDS	WGQGTTLTVSS

Light chain

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
14.2	DILMTQTPLSLPVSLGDQASVSCRSS	QSIVHNDGNTY	LEWYLQKPGQSPKVLIY	KVF	NRFSGVPDRISGSGSGTDFTLTITRVEAEDLGVYYC	FQGSHVPYT	FGGGTKLEIK
14.6	DIQMTQSPSSLSASLGERVSLTCRAS	QEISGY	LSWLQQKPDGTIKRLIY	AAS	TLDSGVPKRFSGSRSGSDYSLTISSLESEDFADYYC	LQYASYPRT	FGGGTKLEIK
14.10	DVLMTQTPLSLPVSLGDQASISCRSS	QSIVHSNGNTY	LEWYLQKPGQSPKLLIY	KVS	NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC	FQGSHVPYT	FGGGTKLEIK
14.13	DVLMTQTPLSLPVSLGDQASISCRSS	QTILHSDGNTY	LEWYLQKPGQSPKLLIY	KVS	TRFSGVPDRFSGSGSGTDFTLKVSRVEAEDLGVYYC	FQGSHVPRT	FGGGTQLEIK
14.15	NVLVTQTPLSLPVSLGDEASISCRSS	QSIVHSNGNTY	LEWYLQKAGQSPKLLIY	KVS	NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC	FQGSHVPYT	FGGGTKLEIK
14.17	DVLMTQTPLSLPVSLGDEASISCKSS	QSIVHSDGNTY	LEWYLQRPGQSPKLLIY	RVF	LRFSGVPDRFAGSGSGTDFTLKISRVEAEDLGIYYC	FQGSHVPRT	FGGGTKLEIT
14.18	DVLLTQTPLSLPVNLGDQASISCRSS	QSIVHSDGYTY	LEWYLQRPGQSPKLLIY	RVY	KRFSGIPDRFSGSGSGMDFTLKISRVEAEDIGVYYC	FQGSYVPRT	FGGGTKLEIK
14.20	DVLMTQTPLSLPVNLGDQASISCRSS	QSIVHSDGYTY	LEWYLQKPGQSPKLLIY	RVS	KRFSGIPDRFSGSGSGMDFTLKISRVEAEDLGVYYC	FQGSYVPRT	FGGGTKLEIK
14.21	DVLLTQTPVSLPVSLGDQGSISCRSS	QSIVHSDGNTY	LEWYLQKPGQSPKLLIY	RVY	IRFSGVPDRFSGSGSGTDFTLKINRVEAEDLGVYYC	FQGTHVPRT	FGGGTKLEIK
14.22	DVLLTQTPLSLPVNLGDQASISCRSS	QTILHSDGYTY	LEWYLQRPGQSPKLLIY	RVY	KRFSGIPDRFRGSGSGMDFTLTISGVEAEDLGIYYC	FQGSYVPRT	FGGGTKLEIK

B

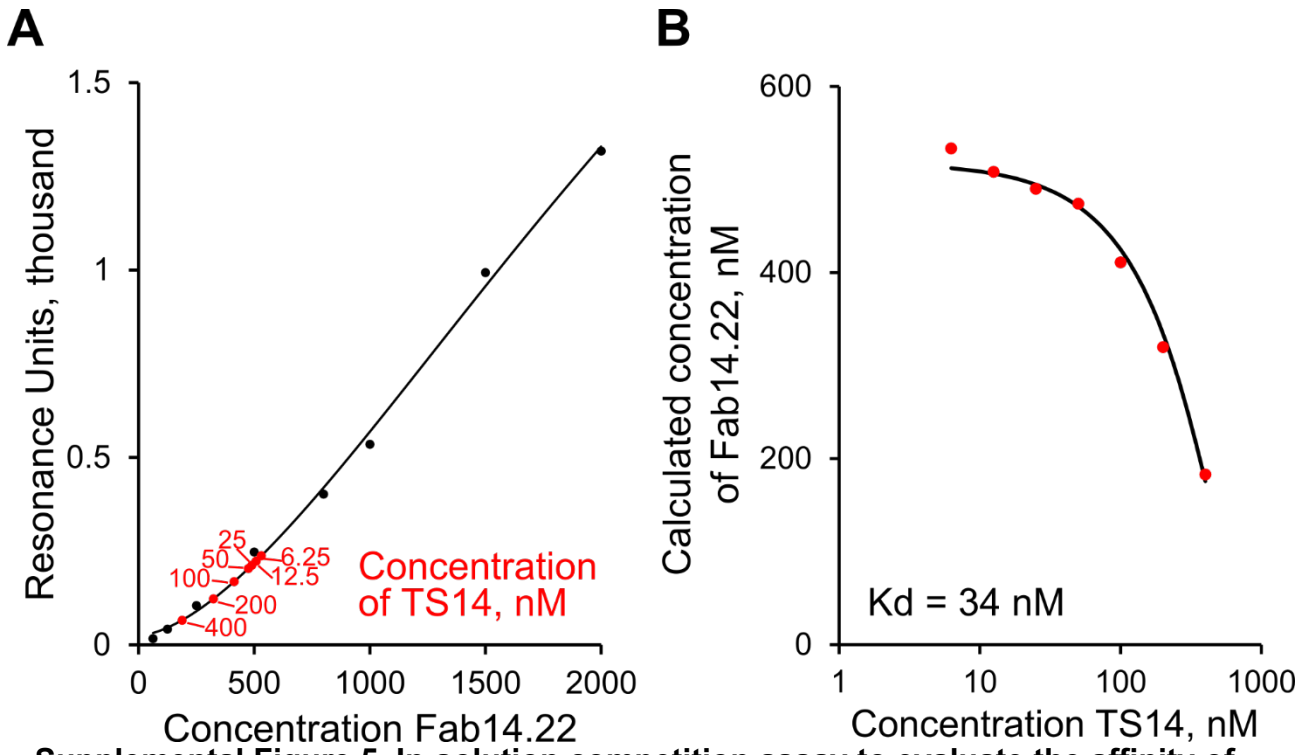
Heavy chain

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
3.1	EVNLEDSGGGLVQPGGSMKLSCVAS	GFTFSTFW	MHWVRQSPEKGLEWVAQ	IKLRSENYAT	YYAESVKGRFTVSRDDSRSSVYLHMNNLRAEDTGIYYC	TSLRRYFVMDY	WGQGTSVTVSS
3.2	EVKLEESGGGLVQPGGSMRLSCVAS	GLTFSNFW	MHWVRQSPEKGLEWVAQ	IKLKSENYAT	HYAESVKGRFTISRDDSKSSVYLQMYNLRPEDTGIYYC	TSLRRYFVLDY	WGQGTSVTVSS
3.3	EVNLEESGGGLVQPGGSIKLSCVAS	GLTFSNFW	MHWVRQSPEKGLEWVAQ	IKLKSENYAT	HYAESVKGRFTISRDDSKSGVYLQMNSLRAEDTGIYYC	TSLRRFFPLDY	WGQGTSVTVSS
3.4	EVTLEESGGGLVQPGGSMKLSCVAS	GFAFSTFW	MHWVRQSPERGLEWVAQ	IKLKSENYAT	HYAGSVNGRFTISRDDSENRVYLQMNNLWTEDTGIYYC	TSLRRFFPMDY	WGQGTSVTVSS
3.5	QVQLQQSDAELVKPGASVKISCKVS	GYTFTDHS	IHWMKERPEQGLEWIGY	FYPRDSST	KYNEKFKGRATLTADKSSSTAYMQLNSLTSEDSAIYFC	ARYSSTSGFVD	WGQGTLVTVSA
3.7	EMNLEESGGGLVHPGGSMKLSCVAS	GFTFSTFW	MHWVRQSPEKGLEWIAQ	IKLRSENFAT	HYAESVKGRFTISRDDSRSSVYLQMNNLGAEDTGIYYC	TSLRRFFIMDY	WGQGTSVTVSS
3.8	EVNLEESGGGLVQPGGSMKLSCVAS	GFTFSTFW	MHWVRQSPEKGLEWVAQ	IKLRSENYAT	HYAESVKGRFTISRDDSRSSVYLQMNNLRTADTGIYYC	TSLRRFFPLDY	WGQGTSVTVSS
3.9	EVTLEESGGGLVQPGGSMKLSCVAS	GFAFSTFW	MHWVRQSPERGLEWVAQ	IKLKSENYAT	HYAGSVNGRFTISRDDSENRVYLQMNNLWTEDTGIYYC	TSLRRFFPMDY	WGQGTSVTVSS
3.10	EVQLVESGGDLVKPGGSLKLSCAAS	GFTFSTYG	MSWVRQTPDKRLEWVAT	ISSGGRYT	NYPDSVKGRFTISRDNAKNTLYLQMRSLKSEDTAMYNC	ARHRGPITTVTHWYFDV	WGTGTTVTVSS
3.11	EVQLQQSMAELVRPGASVKLSCIAS	GFNIKSAY	IHWMKKRPEQGLEWIGR	VDPAKGII	KSAPRFLGKATITADASSNTAYMQLSSLTSEDTAIYYC	ARSFYYGNPYFDY	WGQGTTLTVSS
3.12	EVQLQQSMAELVRPGASVKLSCIAS	GFNIKSAY	IHWMKKRPEQGLEWIGR	VDPAKGII	KSAPRFLGKATITADASSNTAYMQLSSLTSEDTAIYYC	ARSFYYGNPYFDY	WGQGTTLTVSS
3.13	QVQLQQPGTEVVKPGASVKLSCKAS	GYTLISTW	MHWIKQRPGQGLEWIGN	INPRNGGT	NYNEKFKNKATLTVDKSSNTAYMQLNSLTSEDSAVYYC	ARRGDYGSGPAWLAY	WGQGSLVIVSA
3.14	EVQLQQSVAELVRPGASVRLSCTVS	GFNIKNTY	MHWVRRRPEQGLEWIGR	IDPASVIT	KYAPKFQVKATITADTSSNTAYLQLSSLTSEDTAIYFC	ARSFYYGNPYIDY	WGLGTTLTVSS

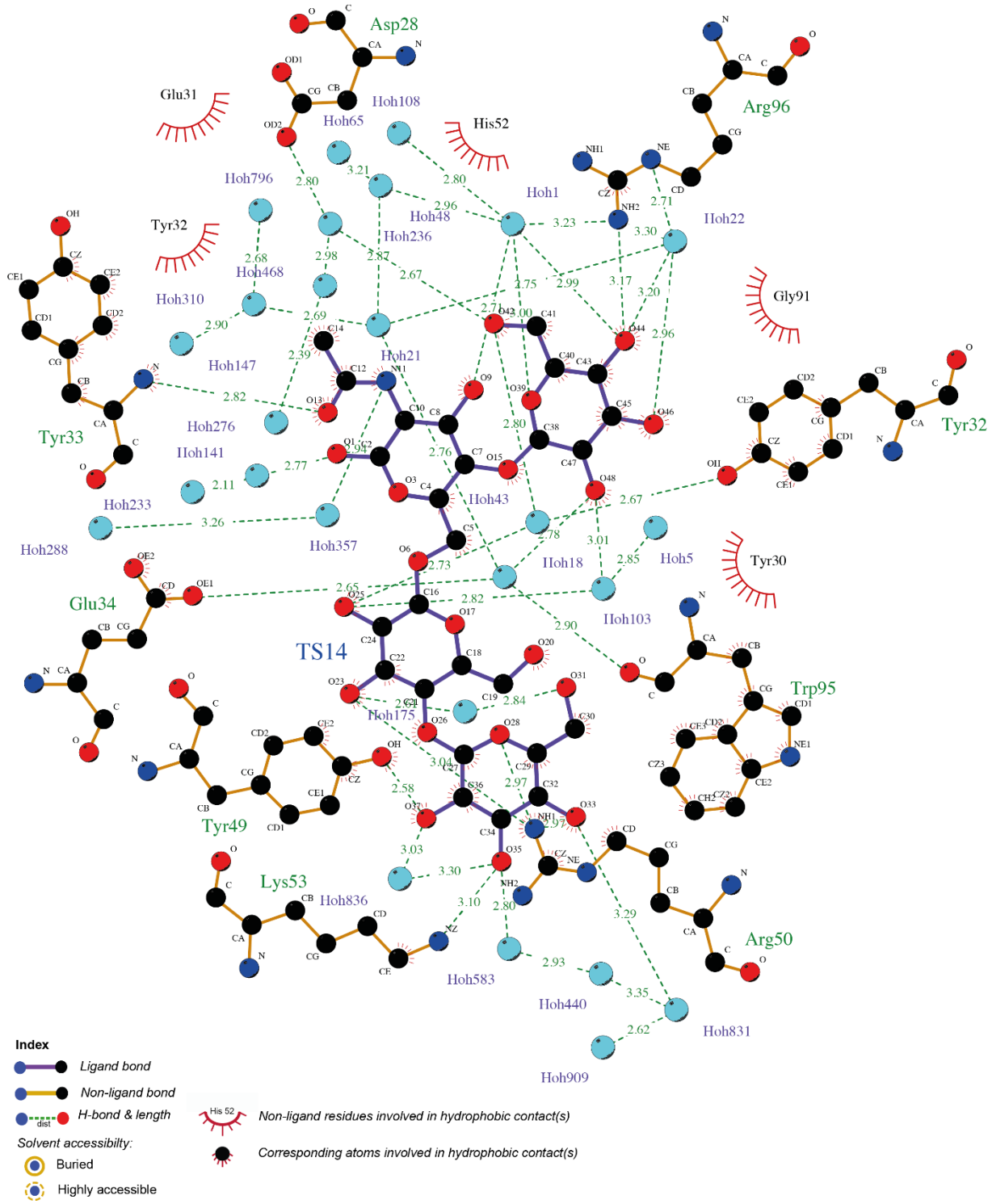
Light chain

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
3.1	DVFMTQTPLTLSVTIGQPASISCRSS	QSLLDY.DGRTY	LNWLLQRPGQSPKRLIY	LVS	KLDSGVPDRFTGSGSGTDFTLRISRVEADDLGIYYC	WQATYFPLT	FGGGTKLELK
3.2	DVVMTQTPLTLSVTIGQPASISCKSS	QSLLDS.DGRTY	LNWLLQRPGQSPKRLIY	LVS	KLDSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYYC	WQATHFPLT	FGAGTKLELK
3.3	DVVMTQTPLTLSVTIGQPTSISCKSS	QSLLDS.DGKTY	LNWLLQRPGQSPKRLIY	LVS	KLDSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYYC	WQATHFPLT	FGAGTKLELK
3.4	DVVMTQTPLTLSITIGQPASISCMSS	QSLLDS.DGKTY	LNWLIQRPGQSPKRLIY	LVS	KLDSGVPDRFSGSGSGTYFTLRISRVETEDLGIYYC	WQATHFPLT	FGAGTKLELK
3.7	DVVMTQTPLTLSVTIGQPASISCKSS	QSLLDT.DGKTY	MGWLLQRPGQSPKRLIF	LVS	KLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYYC	WQSTHFPLT	FGAGTKLELK
3.8	DVVMTQTPFTLSVTIGQPASISCMSS	QSLLDS.DGYTY	LNWLLQRPGQSPKRLIY	LVS	KLDSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYYC	WQSTYFPLT	FGAGTKLELK
3.9	DIVLTQSPASLTVSLGQRATISCRAS	KSVST..SGYSY	MHWYQQKPGQSPKLLIY	LAS	TLQSGVPARVSGSGSGTDFTLNIHPVEEEDAATYYC	QHSRDLPYT	FGGGTKLEVK
3.10	DVLMTQSPLSLPVILGDRASISCRSS	QSIVHS.NGNTY	LEWYLQKPGQSPKLLIY	KVS	NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC	FQGSHVPWT	FGGGTKLEIK
3.11	DVVMTQTPLTLSVTIGQPASISCKSS	QSLLYS.NGKTY	LNWLLQRPGQSPKRLIY	LVS	KLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYYC	VQGTHFPLT	FGAGTKLELK
3.12	DVLMTQTPLSLPVSLGDQASISCRSS	QSIVHS.NGNTY	LEWYLQKPGQSPKLLIY	KVS	NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC	FQGSHPVLT	FGAGTKLELK
3.13	DVVMTQTPFFLPVSLGDQASISCRSS	QSLVHS.NGNTY	FHWYLQKPGQSPKLLIY	KVS	NRFSGVPDRFSGSRSGTDFTLKISRVEAEDLGVYFC	SQSTHVPYT	FGGGTKLEIK

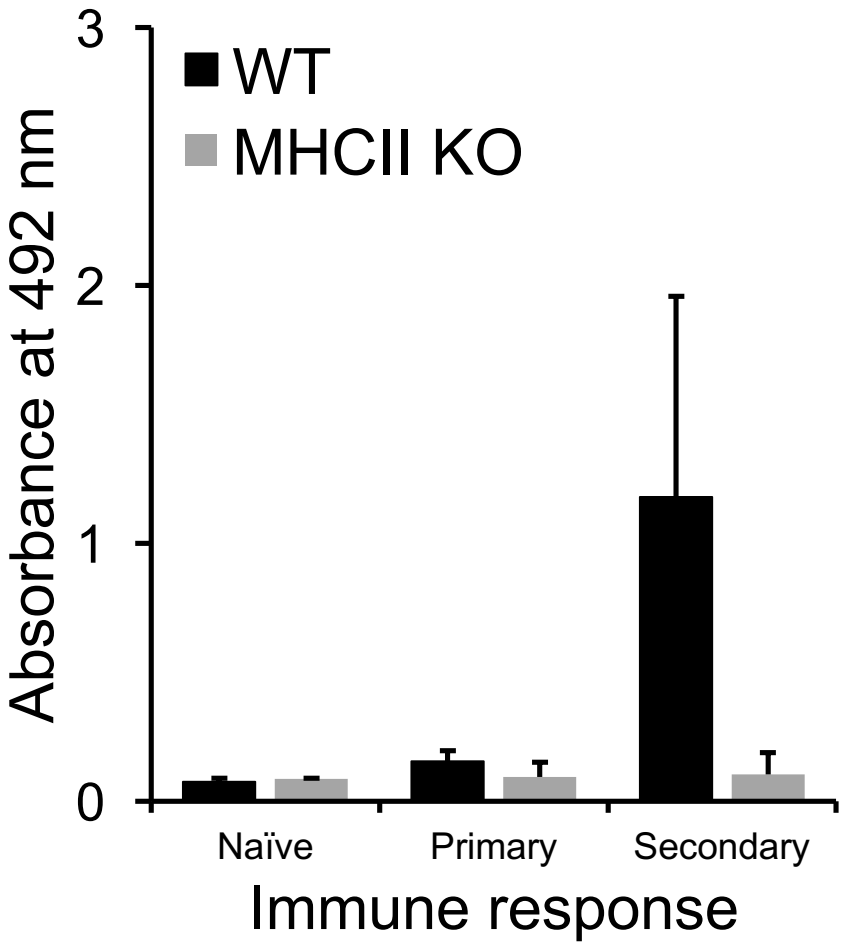
Supplemental Figure 4. Protein sequences of anti-TS14 and anti-TS3 monoclonal antibodies. (**A**) Anti-TS14 antibodies. (**B**) Anti-TS3 antibodies. Sense mutations from the germline sequence are labeled in red. CDRs and FRs are assigned according to the IMGT database. Light chains of antibodies 3.5 and 3.14 were not sequenced due to technical difficulties of sequencing Igλ genes.



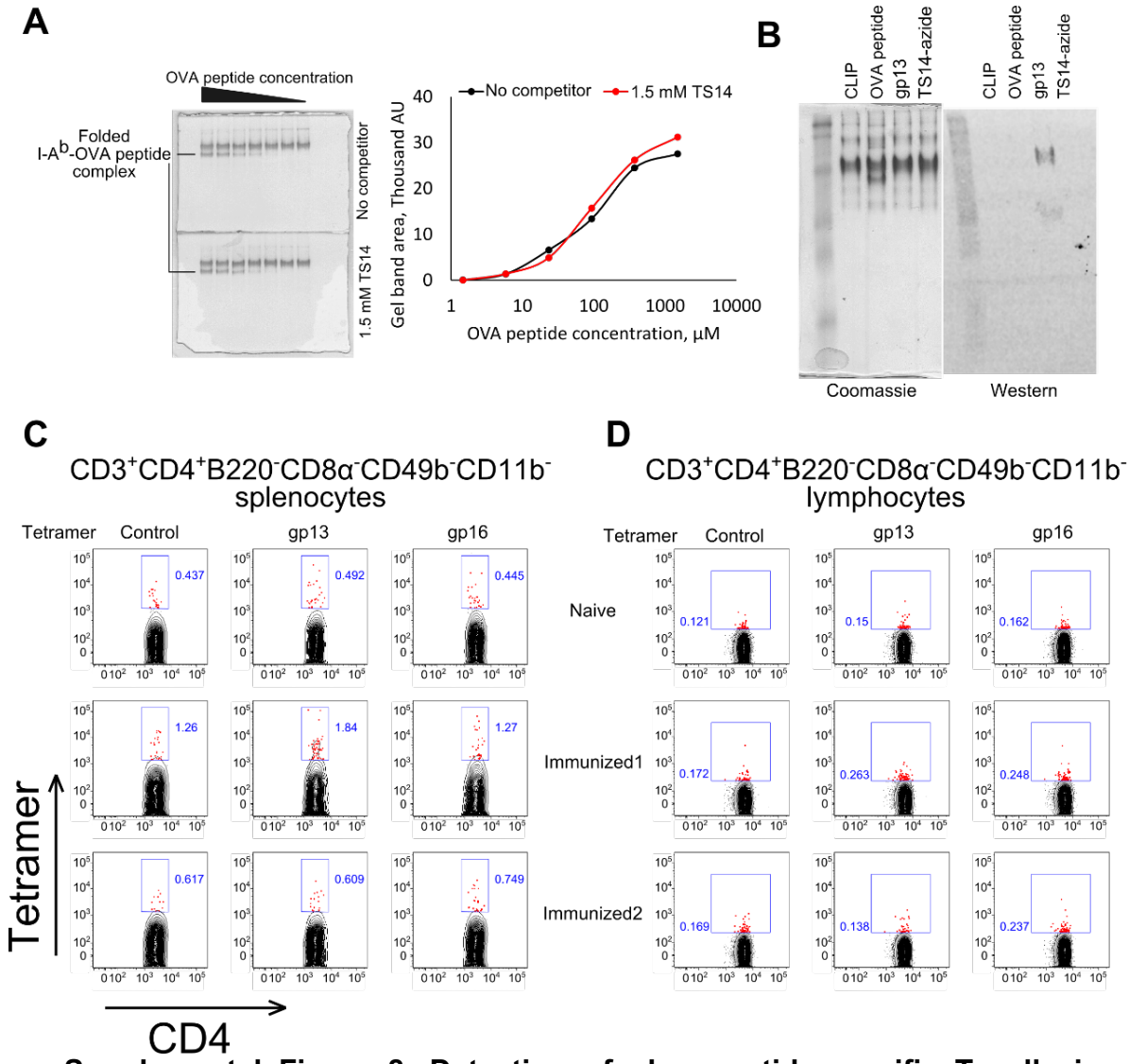
Supplemental Figure 5. In-solution competition assay to evaluate the affinity of Fab14.22 for free TS14. (**A**) Calibration curve based on SPR signal for different concentrations of Fab14.22 binding to TS14-BSA surface (black dots - individual measurements, line - fit). Red dots: signal generated by 500 nM Fab14.22 in the presence of different concentrations of free TS14 (indicated by red numbers). (**B**) Calculated concentration of Fab14.22 plotted against the concentration of free TS14. Black: fit using the Biacore T200 Evaluation Software.



Supplemental Figure 6. The interaction network forming the molecular basis of the nanomolar binding of TS14 tetrasaccharide to Fab14.22. Figure generated using Ligplot (10).

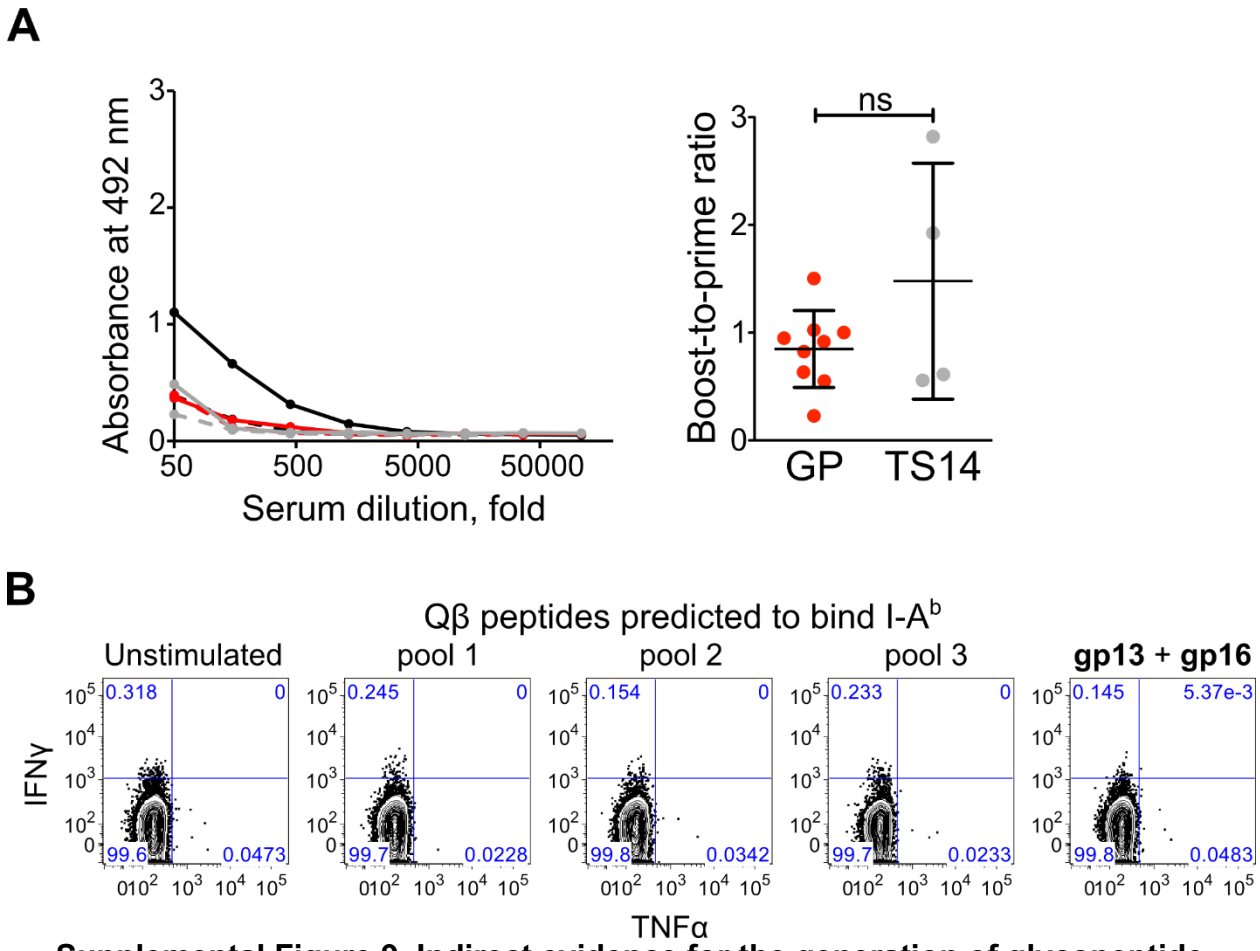


Supplemental Figure 7. IgM response of wild-type and MHC class II$^{-/-}$ mice. Response measured in serum at 1:200 dilution.



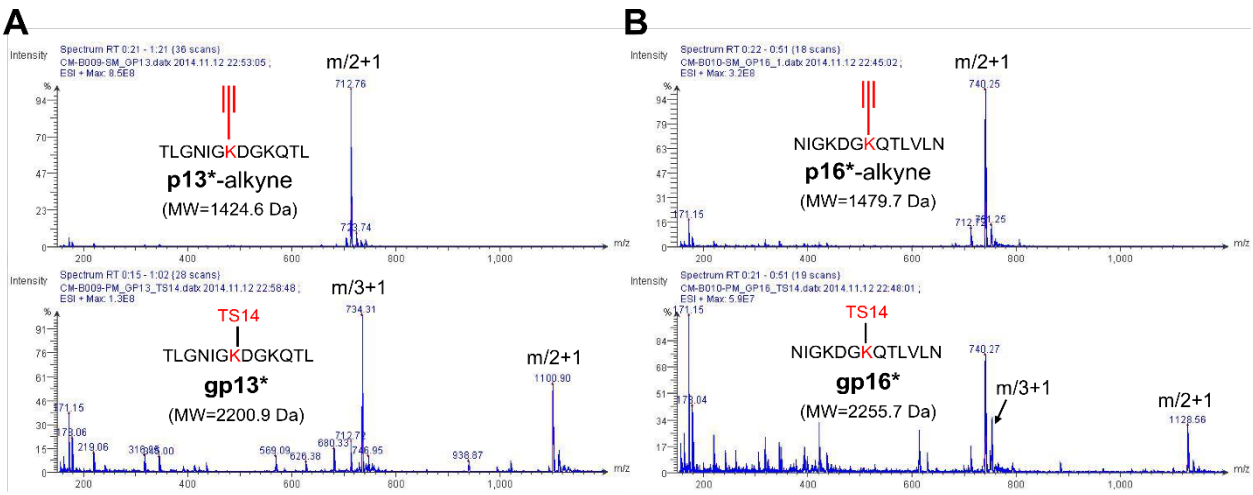
Supplemental Figure 8. Detection of glycopeptide-specific T cells in mice immunized with Qβ-TS14. (**A**) TS14 does not compete with ovalbumin peptide for binding I-Ab. Left: Native SDS-PAGE gel of I-Ab-OVA peptide 323-339 complexes formed at different concentrations of OVA peptide in the presence or absence of 1.5 mM TS14-azide. Right: quantitative determination of the gel band areas from the gels on the left. (**B**) TS14 binding to I-Ab is not detected by western blot with 14.22 monoclonal antibody. (**C**) Detection of glycopeptide-specific CD4 T cells in mouse spleen after secondary immunization. (**D**) Detection of glycopeptide-specific CD4 T cells in mouse lymph nodes

after primary immunization.



Supplemental Figure 9. Indirect evidence for the generation of glycopeptide-specific CD4 T cells after immunization with Qβ-TS14. (**A**) TS14-specific IgMs were measured in post-prime and post-boost sera of immunized mice by ELISA. Left: dilution curves. Dashed lines: post-prime; solid lines: post-boost. Right: ELISA signal at 1:50 dilution generated by post-boost sera was divided by the signal from post-prime sera, resulting in a relative increase in antibody levels after boosting. Each dot represents an individual mouse. Number of mice: 4 for free TS14, 9 for glycopeptide boost, data pooled from two independent experiments. Animals with either post-prime or post-boost IgM response at 1:50 dilution below background of 0.136 were excluded from analysis (1 mouse for TS14 boost, 1 mouse for GP boost). Mean ± s.d. values are reported. (**B**) Intracellular cytokine staining of CD4 T cells in the lymph nodes of a naïve mouse after

5-hour restimulation with the indicated peptides. Data representative of two independent experiments.



Supplemental Figure 10. ESI-MS analysis of peptides and glycopeptides. (**A**) **p13***-alkyne and **gp13***. (**B**) **p16***-alkyne and **gp16*.**

Supplemental Tables

Supplemental Table 1. List of polysaccharides immobilized on the microbial glycan array.

Chart#	BACTERIA / STRAIN
1	Providencia stuartii O49
2	Providencia stuartii O52
3	Pseudomonas aeruginosa O4 (Habs serotype 4)
4	Pseudomonas aeruginosa O1 (Fisher immunotype 4)
5	Pseudomonas aeruginosa O2 (Fisher immunotype 3)
6	Pseudomonas aeruginosa O13 (Sandvik serotype II)
7	Pseudomonas aeruginosa O9 (9a, 9b, 9d)
8	Pseudomonas aeruginosa O6a (Habs serotype 6, fraction IIa)
9	Pseudomonas aeruginosa O6a (Habs serotype 6, fraction IIb)
10	Salmonella typhimurium SL 11881 (Re mutant)
11	Salmonella typhimurium TV 119 (Ra mutant)
12	Salmonella typhimurium SL 684 (Rc mutant)
13	Pseudomonas aeruginosa O10
14	Salmonella typhimurium dodecasaccharide
15	Salmonella enteritidis dodecasaccharide
16	Salmonella typhimurium LPS
17	Serratia marcescens LPS
18	Escherichia coli K235 LPS
19	Escherichia coli O128-B12 LPS
20	Salmonella enterica abortus equi LPS
21	Salmonella typhosa LPS
22	Salmonella enteritidis LPS
23	Shigella bodyii type 2
24	Shigella bodyii type 4
25	Shigella bodyii type 10
26	Shigella dysenteriae type 3
27	Shigella dysenteriae type 8 (batch 12)
28	Shigella dysenteriae type 11
29	Shigella dysenteriae type 13
30	Escherichia coli O29
31	Escherichia coli O40
32	Escherichia coli O106
33	Escherichia coli O130
34	Escherichia coli O148
35	Escherichia coli O150
36	Escherichia coli O180
37	Proteus mirabilis O3a, 3c (G1)
38	Proteus mirabilis O8 (TG326)
39	Proteus mirabilis O10 (HJ4320)
40	Proteus mirabilis O29a, 29b (2002)
41	Proteus mirabilis O50 (TG332)
42	Proteus mirabilis O54a, 54b (10704)
43	Proteus mirabilis O57 (TG319)
44	Proteus penneri O8 (106)
45	Proteus penneri O64a, 64b, 64d (39)
46	Proteus penneri O66 (2)
47	Proteus penneri O69 (25)
48	Proteus penneri O71 (42)
49	Proteus penneri O72a, 72b (4)
50	Pseudomonas aeruginosa O2 (2a),2d,2f
51	Pseudomonas aeruginosa O2 2a,2b
52	Pseudomonas aeruginosa O2 2a,2b,2e
53	Pseudomonas aeruginosa O2 2a,2d
54	Pseudomonas aeruginosa O2 Immuno 7
55	Pseudomonas aeruginosa O3 3a,3b
56	Pseudomonas aeruginosa O3 3a,3b,3c
57	Pseudomonas aeruginosa O3 3a,3d
58	Pseudomonas aeruginosa O4 4a,4c
59	Pseudomonas aeruginosa O6 6a
60	Pseudomonas aeruginosa O6 6a,6c

61	Pseudomonas aeruginosa O6 Immuno 1
62	Pseudomonas aeruginosa O7 7a,7b,7c
63	Pseudomonas aeruginosa O7 7a,7b,7d
64	Pseudomonas aeruginosa O7 7a,7d
65	Pseudomonas aeruginosa O10 10a,10b
66	Pseudomonas aeruginosa O10 10a,10c
67	Pseudomonas aeruginosa O11 11a,11b
68	Pseudomonas aeruginosa O12 12
69	Pseudomonas aeruginosa O13 13a,13c
70	Pseudomonas aeruginosa O14 14
71	Pseudomonas aeruginosa O15 15
72	Proteus vulgaris O1 (18984)*
73	Proteus vulgaris O4 (PrK 9/57)
74	Proteus vulgaris O12 (PrK 25/57)
75	Proteus vulgaris O13 (8344)
76	Proteus vulgaris O15 (PrK 30/57)
77	Proteus vulgaris O17 (PrK 33/57)
78	Proteus vulgaris O19a (PrK 37/57)
79	Proteus vulgaris O21 (PrK 39/57)*
80	Proteus vulgaris O22 (PrK 40/57)
81	Proteus vulgaris O25 (PrK 48/57)
82	Proteus vulgaris O34 (4669)*
83	Proteus vulgaris O37a,b (PrK 63/57)
84	Proteus vulgaris O37a,c (PrK 72/57)
85	Proteus vulgaris O44 (PrK 67/57)
86	Proteus vulgaris O45 (4680)
87	Proteus vulgaris O53 (TG 276-10)
88	Proteus vulgaris O54a,54c (TG 103)
89	Proteus vulgaris O55 (TG 155)
90	Proteus vulgaris O65 (TG 251)
91	Proteus mirabilis O6 (PrK 14/57)
92	Proteus mirabilis O11 (PrK 24/57)
93	Proteus mirabilis O13 (PrK 26/57)
94	Proteus mirabilis O14a,14b (PrK 29/57)
95	Proteus mirabilis O16 (4652)
96	Proteus mirabilis O17 (PrK 32/57)
97	Proteus mirabilis O23a,b,d (PrK 42/57)
98	Proteus mirabilis O26 (PrK 49/57)
99	Proteus mirabilis O27 (PrK 50/57)
100	Proteus mirabilis O28 (PrK 51/57)
101	Proteus mirabilis O29a (PrK 52/57)
102	Proteus mirabilis O40 (10703)
103	Proteus mirabilis O41 (PrK 67/57)
104	Proteus mirabilis O51 (19011)*
105	Proteus mirabilis O74 (10705, OF)
106	Proteus mirabilis O75 (10702, OC)
107	Proteus mirabilis O77 (3 B-m)
108	Proteus penneri O31a (26)
109	Proteus penneri O52 (15)
110	Proteus penneri O58 (12)
111	Proteus penneri O59 (9)
112	Proteus penneri O61 (21)
113	Proteus penneri O62 (41)
114	Proteus penneri O63 (22)
115	Proteus penneri O64a,b,c (27)
116	Proteus penneri O65 (34)
117	Proteus penneri O67 (8)
118	Proteus penneri O68 (63)
119	Proteus penneri O70 (60)
120	Proteus penneri O73a,b (103)
121	Proteus myxofaciens O60
122	Proteus O56 (genomospecies 4)
123	Providencia stuartii O4
124	Providencia stuartii O18
125	Providencia stuartii O20*
126	Providencia stuartii O43

127	Providencia stuartii O44
128	Providencia stuartii O47
129	Providencia stuartii O47, Core 9
130	Providencia stuartii O49, Core 1
131	Providencia stuartii O57
132	Providencia alcalifaciens O5
133	Providencia alcalifaciens O6*
134	Providencia alcalifaciens O19
135	Providencia alcalifaciens O19
136	Providencia alcalifaciens O19
137	Providencia alcalifaciens O21
138	Providencia alcalifaciens O23
139	Providencia alcalifaciens O27
140	Providencia alcalifaciens O29
141	Providencia alcalifaciens O30
142	Providencia alcalifaciens O32
143	Providencia alcalifaciens O36*
144	Providencia alcalifaciens O39
145	Providencia rustigianii O14
146	Providencia rustigianii O16
147	Providencia rustigianii O34
148	Yersinia pestis, KM260(11)-Δ0187
149	Yersinia pestis, KM260(11)-Δ0187
150	Yersinia pestis, KM260(11)-Δrfe
151	Yersinia pestis, KM260(11)-Δrfe
152	Yersinia pestis, 1146-25
153	Yersinia pestis 1146-25
154	Yersinia pestis, 1146-37
155	Yersinia pestis, 1146-37
156	Yersinia pestis, 0KM218-37
157	Yersinia pestis, KM218-37
158	Yersinia pestis, KM218-25
159	Yersinia pestis, KM218-25
160	Yersinia pestis, KM260(11)-ΔpmrF
161	Yersinia pestis, KM260(11)-ΔpmrF
162	Yersinia pestis, KM260(11)-Δ0186
163	Yersinia pestis, KM260(11)-Δ0186
164	Yersinia pestis, KM260(11)-ΔwaaQ
165	Yersinia pestis, KM260(11)-ΔwaaQ
166	Yersinia pestis, KM260(11)-ΔwaaL
167	Yersinia pestis, KM260(11)-25
168	Yersinia pestis, KM260(11)-25
169	Yersinia pestis, KM260(11)-37
170	Yersinia pestis, KIMD1-37
171	Yersinia pestis, KIMD1-25
172	Yersinia pestis, 11M-25
173	Yersinia pestis, 11M-37
174	Proteus mirabilis O23a, 23b, 23c (CCUG 10701)
175	Proteus vulgaris O24 (PrK 47/57)
176	Yersinia pestis KM260(11)-6C
177	Yersinia pestis 260(11)-37C-186
178	Yersinia pestis 260(11)-37C-187
179	Yersinia pestis 260(11)-37C-416
180	Yersinia pestis 260(11)-37C-417
181	Yersinia pestis P-1680-25C
182	Yersinia pestis P-1680-37C
183	Yersinia pestis I-2377-25C
184	Yersinia pestis I-2377-37C
185	Francisella novicida OPS
186	Francisella tularensis OPS
187	Klebsiella O1 OPS
188	Klebsiella O2a OPS
189	Klebsiella O2ac OPS
190	Klebsiella O3 OPS
191	Klebsiella O4 OPS
192	Klebsiella O5 OPS

193	Klebsiella O8 OPS
194	Klebsiella O12 OPS
195	Shigella boydii type 1
196	Shigella boydii type 3
197	Shigella boydii type 5
198	Shigella boydii type 9
199	Shigella boydii type 11
200	Shigella boydii type 12
201	Shigella boydii type 15
202	Shigella boydii type 16
203	Shigella boydii type 17
204	Shigella boydii type 18
205	Escherichia coli O49
206	Escherichia coli O52
207	Escherichia coli O58
208	Escherichia coli O61
209	Escherichia coli O73
210	Escherichia coli O112ab
211	Escherichia coli O118
212	Escherichia coli O125
213	Escherichia coli O151
214	Escherichia coli O168
215	Shigella dysenteriae type 2
216	Shigella dysenteriae type 4
217	Shigella dysenteriae type 5
218	Shigella dysenteriae type 6 SR-strain
219	Shigella dysenteriae type 7
220	Shigella dysenteriae type 8 (Russian)
221	Shigella dysenteriae type 9
222	Escherichia coli O111:B4 LPS
223	Escherichia coli O26:B6 LPS
224	Escherichia coli O55:B5 LPS
225	Escherichia coli O127:B8 LPS
226	Streptococcus pneumoniae type 1 (Danish type 1)
227	Streptococcus pneumoniae type 2 (Danish type 2)
228	Streptococcus pneumoniae type 3 (Danish type 3)
229	Streptococcus pneumoniae type 4 (Danish type 4)
230	Streptococcus pneumoniae type 5 (Danish type 5)
231	Streptococcus pneumoniae type 8 (Danish type 8)
232	Streptococcus pneumoniae type 9 (Danish type 9N)
233	Streptococcus pneumoniae type 12 (Danish type 12F)
234	Streptococcus pneumoniae type 14 (Danish type 14)
235	Streptococcus pneumoniae type 17 (Danish type 17F)
236	Streptococcus pneumoniae type 19 (Danish type 19F)
237	Streptococcus pneumoniae type 20 (Danish type 20)
238	Streptococcus pneumoniae type 22 (Danish type 22F)
239	Streptococcus pneumoniae type 23 (Danish type 23F)
240	Streptococcus pneumoniae type 26 (Danish type 6B)
241	Streptococcus pneumoniae type 34 (Danish type 10A)
242	Streptococcus pneumoniae type 43 (Danish type 11A)
243	Streptococcus pneumoniae type 51 (Danish type 7F)
244	Streptococcus pneumoniae type 54 (Danish type 15B)
245	Streptococcus pneumoniae type 56 (Danish type 18C)
246	Streptococcus pneumoniae type 57 (Danish type 19A)
247	Streptococcus pneumoniae type 68 (Danish type 9V)
248	Streptococcus pneumoniae type 70 (Danish type 33F)
249	Yersinia pestis KM218-6C
250	Yersinia pestis KM260(11)-yjhW-6C
251	Yersinia pestis KM260(11)-wabD/waaL
252	Yersinia pestis KM260(11)-wabC/waaL
253	Yersinia pseudotuberculosis 85pCad-37C
254	Yersinia pseudotuberculosis 85pCad-20C
255	Yersinia pseudotuberculosis O:2a
256	Yersinia pseudotuberculosis O:2a-dhmA
257	Yersinia pseudotuberculosis O:2c
258	Yersinia pseudotuberculosis O:3

259	Yersinia pseudotuberculosis O:4b
260	Proteus vulgaris O2 (OX2)
261	Proteus mirabilis O3ab (S1959)
262	Proteus mirabilis O5 (PrK 12/57)
263	Proteus mirabilis O9 (PrK 18/57)
264	Proteus mirabilis O11 (9B-m)
265	Proteus penneri O17 (16)
266	Proteus mirabilis O18 (PrK 34/57)
267	Proteus mirabilis O20 (PrK 38/57)
268	Proteus penneri O31ab (28)
269	Proteus mirabilis O33 (D52)
270	Proteus mirabilis O43 (PrK 69/57)
271	Proteus vulgaris O47 (PrK 73/57)
272	Proteus mirabilis O49 (PrK 75/57)
273	Proteus mirabilis O54ab (OE)
274	Proteus penneri O73ac (75)
275	Proteus vulgaris O76 (HSC438)
276	Shigella flexneri type 1a
277	Shigella flexneri type 1b
278	Shigella flexneri type 2a
279	Shigella flexneri type 2b
280	Shigella flexneri type 3a
281	Shigella flexneri type 3b
282	Shigella flexneri type 4a
283	Shigella flexneri type 4b
284	Shigella flexneri type 5b
285	Shigella flexneri type 6a
286	Shigella flexneri type 6
287	Shigella flexneri type X
288	Shigella dysenteriae type 1
289	Shigella boydii type 6
290	Shigella boydii type 7
291	Shigella boydii type 8
292	Shigella boydii type 13
293	Shigella boydii type 14
294	Escherichia coli O71
295	Escherichia coli O85
296	Escherichia coli O99
297	Escherichia coli O145
298	Escherichia coli O107
299	Salmonella enterica O17
300	Salmonella enterica O28
301	Salmonella enterica O47
302	Salmonella enterica O55
303	Escherichia coli K92
304	Escherichia coli K5
305	Escherichia coli K13
306	Neisseria meningitidis Group C
307	Davanat
308	Laminarin
309	Yeast Mannan
310	Escherichia coli O86
311	Galactomannan DAVANAT (160102) Pro-Pharmacenti
312	Yeast Mannan Sigma M-3640
313	1-2 Mannan Acetobacter methanolicus MB135

Modified from (15).

Supplemental Table 2. Gene usage and mutations of anti-TS14 and anti-TS3 monoclonal antibodies.

Name	Isotype	Number of mutations, heavy chain	Number of mutations, light chain	Number of mutations in CDR, heavy chain	Number of mutations in CDR, light chain	CDRH3 length	VH	DH	JH	VL κ	JL
14.2	IgG1	17	9	8	3	11	1-58	1-1	1	1-117	2
14.6	IgM	5	0	2	n/a	13	1-64	2-1	1	9-124	1
14.10	IgG1	11	0	8	n/a	12	1-58	1-1	1	1-117	2
14.13	IgG2b	10	5	5	3	5	1-22	-0-	2	1-117	1
14.15	IgG2c	11	4	5	0	11	1-58	2-2	1	1-117	1
14.17	IgG2b	13	9	5	3	5	1-22	n/a	2	1-117	1
14.18	IgG2b	15	12	7	5	5	1-22	n/a	2	1-117	1
14.20	IgG2b	18	8	7	4	5	1-22	n/a	2	1-117	1
14.21	IgG2b	12	9	5	4	5	1-64	n/a	2	1-117	1
14.22	IgG2b	22	17	10	7	5	1-22	n/a	2	1-117	1
3.1	IgG1	14	10	7	4	11	6-3	1-2	4	1-135	5
3.2	IgG3	11	2	7	2	11	6-3	1-2	4	1-135	5
3.3	IgG3	11	2	6	1	11	6-3	2-2	4	1-135	5
3.4	IgG2c	16	8	6	1	11	6-3	1-2	4	1-135	5
3.5	IgG3	11	n.d.	8	n.d.	11	1-78	1-3	3	n.d.	n.d.
3.7	IgG2c	14	7	7	2	11	6-3	1-2	4	1-135	5
3.8	IgG3	11	5	7	3	11	6-3	1-2	4	1-135	5
3.9	IgG2c	16	6	6	1	11	6-3	1-2	4	3-12	2
3.10	IgG1	5	3	2	0	17	5-6	1-1	1	1-117	1
3.11	IgG2c	16	0	6	n/a	13	14-3	2-1	2	1-133	5
3.12	IgG2c	16	0	6	n/a	13	14-3	2-1	2	1-117	5
3.13	IgG1	11	4	4	0	15	1-53	1-1	3	1-110	2
3.14	IgG2b	12	n.d.	5	n.d.	13	14-3	1-1	2	n.d.	n.d.

n.d. – not determined due to difficulties in sequencing Igλ genes.

Supplemental Table 3. Characterization of anti-TS3 monoclonal antibodies isolated from Qβ-TS3-immunized mice.

1:1 binding kinetic model				
Name	Isotype	Avidity, nM, BSA-TS3	k_{on}, 10^3 $M^{-1}*s^{-1}$	k_{off}, 10^{-6} s^{-1}
3.1	IgG1	<0.1	10	<0.1
3.2	IgG3	<0.1	0.7	<0.1
3.3	IgG3	0.4	11	5
3.4	IgG2c	<0.1	1.8	<0.1
3.7	IgG2c	1.9	0.14	0.27
3.8	IgG3	<0.1	180	2.6
3.9	IgG2c	12	2.7	33
3.10	IgG1	3600	0.08	280
3.11	IgG2c	9	96	870

Bivalent analyte kinetic model					
Name	Isotype	$k_{on}1$, 10^4 $M^{-1}*s^{-1}$	$k_{off}1$, 10^{-3} s^{-1}	$k_{on}2$, 10^{-6} RU^{-1}	$k_{off}2$, 10^{-4} s^{-1}
3.5	IgG3	0.8	14	3.1	5.4
3.12	IgG2c	2.3	1.8	2000	300
3.13	IgG1	0.2	3.7	37	4.8
3.14	IgG2b	3.6	6.1	14	3.8

Biacore T200 Evaluation software was used to estimate kinetic and affinity constants

Supplemental Table 4. Crystallographic data collection and refinement statistics for Fab14.22 unliganded and in complex with TS14.

	Fab14.22-tetrasaccharide complex	Fab14.22
Data collection		
Beamline	APS 23 ID-B	APS 23 ID-D
Detector	MARMosaic300	Pilatus6M
Wavelength (Å)	1.03317	1.03321
Space group	C2	$P2_1$
Unit cell (*a, b,* and *c*; Å)	125.27, 74.74, 120.30	120.61, 75.99, 122.94
(α, β and γ; °)	90.0, 100.6, 90.0	90.0, 100.6, 90.0
Resolution range* (Å)	47.19 - 1.75	49.74 - 2.21
No. of total reflections	288,284 (14,607)	393,318 (16,194)
No. of unique reflections	106,772 (5,410)	109,255 (5,224)
Redundancy*	2.7 (2.7)	3.6 (3.1)
Completeness* (%)	97.0 (98.3)	99.4 (95.3)
R_{sym}[a]*	6.5 (63.9)	13.3 (68.0)
R_{pim}[b]*	4.3 (44.9)	8.1 (43.7)
$\langle I \rangle / \langle \sigma \rangle$*	11.4 (1.4)	6.1 (1.6)
$CC_{1/2}$[c]	92.7 (68.2)	90.2 (67.2)
Solvent content (%)	56.8	57.7
Refinement		
Reflections used for refinement (R_{free})	106,745	109,105
R_{cryst}[d] (%)	16.9	20.4
R_{free}[e] (%)	20.3	23.8
Model components (asymmetric unit)		
Fabs	2	4
TS14	2	-
Waters	931	761
PO_4 ions	30	-
SO_4 ions	-	9
Glycerol	8	4
B-values ($Å^2$)		
Wilson B	22.3	37.5
Overall	31.2	44.8
Protein	29.5	44.6
Glycan	28.0	-
Root mean square deviation from ideal values		
Bond lengths (Å)	0.015	0.002
Bond angles (°)	1.4	0.6
Ramachandran values		
Most favored regions (%)	98.2	97.1
Additional allowed regions (%)	1.6	2.8
Disallowed regions (%)	0.2	0.1

* Values in parentheses correspond to the highest resolution shells

[a] $R_{sym} = \Sigma_{hkl} \Sigma_{j=1,N} | \langle I_{hkl} \rangle - I_{hklj} | / \Sigma_{hkl} \Sigma_{j=1,N} | I_{hklj} |$, where the outer sum (hkl) is taken over the unique reflections

[b] $R_{pim} = \Sigma_{hkl} [1/(N-1)]^{1/2} \Sigma_{i=1,N} | I_{hkli} - \langle I_{hkl} \rangle | / \Sigma_{hkl} \Sigma_{i=1,N} | I_{hkli} |$

[c] $CC_{1/2}$ = Pearson Correlation Coefficient between two random half datasets

[d] $R_{cryst} = \Sigma_{hkl} || F_{o,hkl} | - k | F_{c,hkl} || / \Sigma_{hkl} | F_{o,hkl} |$, where $|F_{o,hkl}|$ and $|F_{c,hkl}|$ are the observed and calculated structure factor amplitudes, respectively

[e]R_{free} , as for R_{cryst}, but for a set of reflections (5% of total) omitted from refinement

Supplemental Table 5. Interactions between TS14 and Fab14.22 in the crystal structure.

		Polar contacts	Hydrophobic contacts
Heavy chain	CDR1	Tyr33	Glu31, Tyr32
	CDR2		His52
Light chain	CDR1	Asp28, Tyr32, Tyr33, Glu34	Tyr30
	FR2	Tyr49	
	CDR2	Arg50, Lys53	
	FR3	Gly91	
	CDR3	Trp95, Arg96	Gly91

Protein residues numbered according to Kabat numbering system.

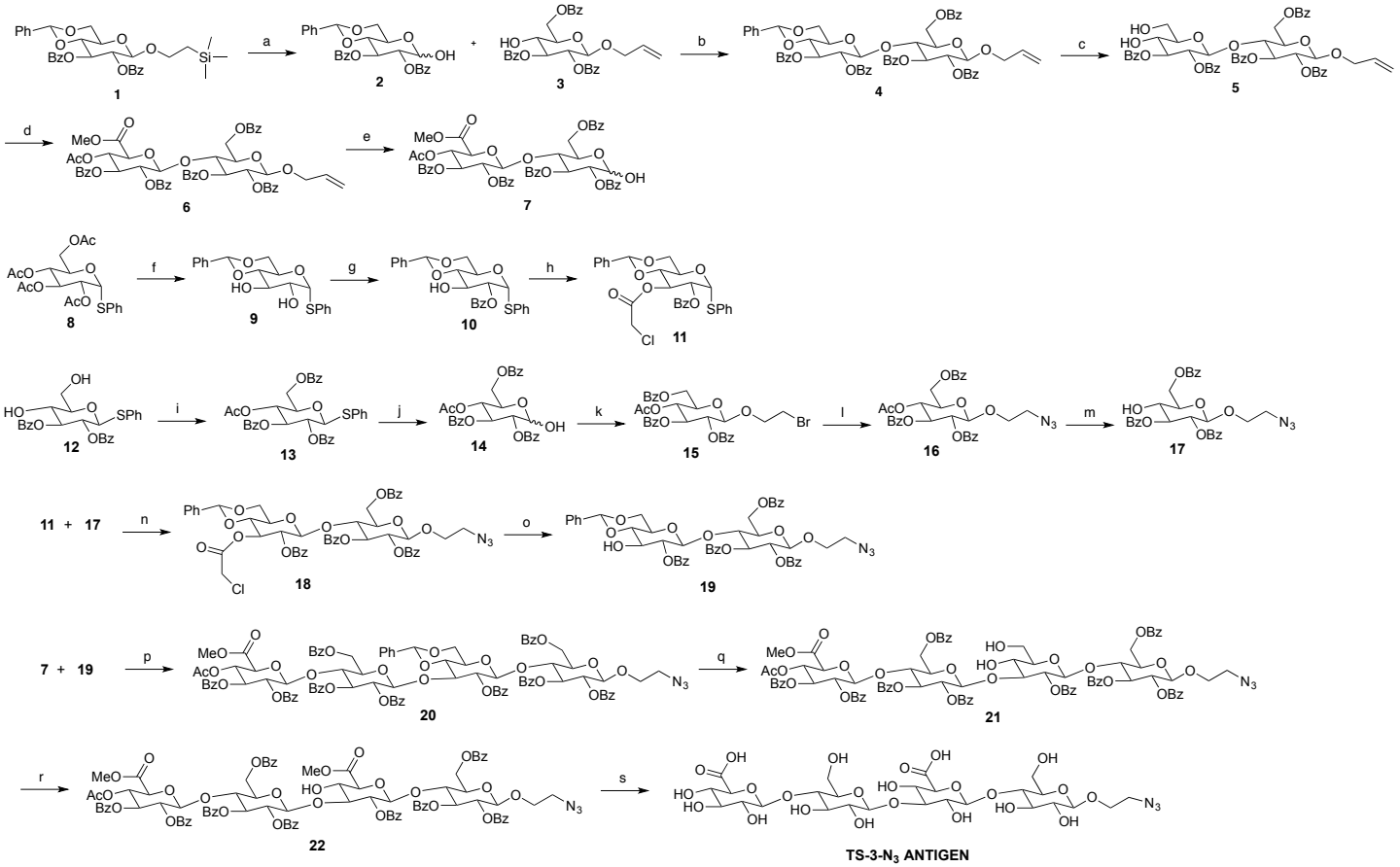
Supplemental Table 6. Sequences of the peptides used in cell assays. Qβ coat protein residues are numbered after omitting initiator Met cleaved during protein processing. Bold – lysine residue modified with TS14 in glycopeptides.

Peptide name	Peptide sequence	Qβ CP residues	Assays used
p13	LGNIG**K**DGKQT	8-18	Glycopeptides for tetramer staining and ICS
p13*	TLGNIG**K**DGKQTL	7-19	Glycopeptides for mouse immunization
p16	IGKDG**K**QTLVL	11-21	Glycopeptides for tetramer staining and ICS
p16*	NIGKDG**K**QTLVLN	10-22	Glycopeptides for mouse immunization
p30-44	NGVASLSQAGAVPAL	30-44	ICS staining: peptide pool 1
p31-45	GVASLSQAGAVPALE	31-45	ICS staining: peptide pool 1
p32-46	VASLSQAGAVPALEK	32-46	ICS staining: peptide pool 1
p44-58	LEKRVTVSVSQPSRN	44-58	ICS staining: peptide pool 2
p45-59	EKRVTVSVSQPSRNR	45-59	ICS staining: peptide pool 2
p46-60	KRVTVSVSQPSRNRK	46-60	ICS staining: peptide pool 2
p107-121	FVRTELAALLASPLL	107-121	ICS staining: peptide pool 3
p108-122	VRTELAALLASPLLI	108-122	ICS staining: peptide pool 3
p109-123	RTELAALLASPLLID	109-123	ICS staining: peptide pool 3

Compound synthesis

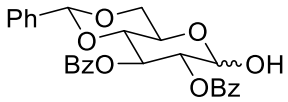
Glycan synthesis

Materials and General Methods. ^{1}H NMR and ^{13}C NMR were recorded on Varian Unity 500 MHz or Varian Unity 300 MHz instruments. Mass spectrometric data were obtained on JEOL SX 102 A spectrometer or Agilent 1100 series spectrometer. All solvents were dried using activated alumina columns. Chemicals were obtained from Sigma and Aldrich and were used as received unless otherwise noted.



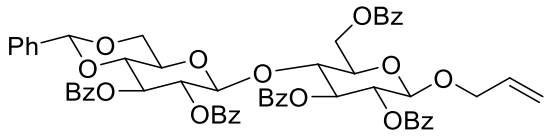
Reagents: a: i) $TsOH{\cdot}H_2O$, MeOH, DCM, 60-70 °C; ii) $PhCH(OMe)_2$, DMF, 60 °C, 85% yield. b: i) K_2CO_3, CCl_3CN, DCM, 54% yield; ii) DCM, TMSOTf, 4 Å molecular sieves, 60% yield. c: MeOH, DCM, $TsOH{\cdot}H_2O$, 81% yield. d: i) BAIB, TEMPO, DCM, H_2O; ii) diazomethane, Et_2O; iii) Ac_2O, Et_3N, DMAP, 82% yield for 3 steps. e: i) chlorotris(triphenylphosphine)rhodium(I), EtOH, toluene, reflux; ii) NIS, THF, H_2O, 82% yield. f: i) NaOMe, THF, MeOH; ii) H^+ form resin, DMF, $PhCH(OMe)_2$, $TsOH{\cdot}H_2O$, 84% yield for 2 steps. g: BzCl, pyridine, 75% yield. h: DCM, pyridine, chloroacetic anhydride, 92% yield. i: i) DCM, pyridine, BzCl; ii) Ac_2O, 65% yield for 2 steps. j: acetone, water, NBS, 85% yield. k: i) K_2CO_3, DCM, CCl_3CN; DCM, TMSOTf, 3 Å molecular sieves, 89% yield. l: DMF, NaN_3, 97% yield. m: THF, MeOH, AcCl, 78% yield. n: DCM, NIS, TMSOTf, 4 Å molecular sieves, 57% yield. o: toluene, ethanol, DABCO, 83% yield. p: i) 7, K_2CO_3, DCM, CCl_3CN; ii) 4 Å molecular sieves, DCM, TMSOTf, 78% yield. q: DCM, MeOH, $TsOH.H_2O$, 88% yield. r: BAIB, TEMPO, DCM, H_2O; diazomethane in Et_2O, DCM, 73% yield. s: NaOMe, THF, MeOH, H_2O, 64% yield.

Scheme 1. Synthesis of TS-3-N_3 Antigen



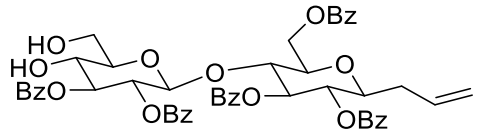
Preparation of compound 2: To a solution of compound **1** (1.5 g, 2.6 mmol) in a mixture of DCM (20 mL) and methanol (15 mL) was added $TsOH{\cdot}H_2O$ (49 mg, 0.26 mmol, 0.1 eq). The reaction was stirred at room temperature for 5 h. The solvent was then removed under vacuum. The crude mixture was subsequently dissolved in dry DMF (20 mL) and stirred with benzaldehyde dimethyl acetal (1 mL) at 60 °C. After 30 min, the

reaction was stopped by addition of triethyl amine (2 mL). The solvent was removed by evaporation under vacuum. The resulting mixture was subjected to flash column chromatography (SiO_2), using 50% ethyl acetate in hexanes as eluent, affording the desired product as a clear oil (1.05 g, 85% yield). ^{1}H NMR (alpha anomer, 500 MHz, $CDCl_3$, ppm): δ = 8.02-7.30 (m, 15 H), 6.16 (t, J = 10.0 H, 1 H), 5.69 (d, J = 3.0 Hz, 1 H, anomeric), 5.58 (s, 1 H), 5.32 (dd, J = 10.5, 4.0 Hz, 1 H), 4.36 (m, 1 H), 4.33 (t, J = 5.0 Hz, 1 H), 3.90 (dd, J = 9.0 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm); 166.15, 165.84, 136.93, 133.61, 133.11, 129.96, 129.81, 129.77, 128.84, 128.49, 128.33, 128.23, 128.20, 126.21, 101.63, 91.19, 79.43, 72.86, 69.49, 68.96, 62.59. HRMS (ESI) calcd for $C_{27}H_{24}O_8$ $[M+H]^+$: 477.1543; found: 477.1519.



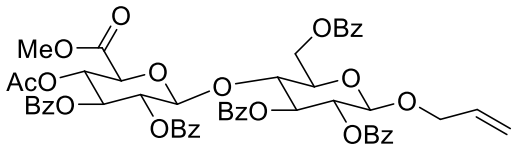
Compound 4: To a solution of compound **2** (270 mg, 0.567 mmol) in DCM (15 mL) was added anhydrous K_2CO_3 (500 mg), and CCl_3CN (2 mL). The mixture was stirred at room for 5 h then solids were removed by filtration, and the filtrate was conducted under vacuum. The concentrated mixture was subjected to silica gel column chromatography using 25 % EtOAc in hexanes, affording 190 mg (54% yield) of activated donor as a clear oil, which was then mixed with acceptor **3** (179.3 mg, 0.337 mmol) in DCM (6 mL). The reaction mixture was stirred at ambient temperature for 1 h, followed by addition of TMSOTf (10 µL). The mixture was allowed to stir for 5 h before Et_3N (0.1 mL) was added. Silica gel column chromatography was used for purification of products and 75 % EtOAc in hexanes was used as eluent, affording 180 mg (60 % yield) of the disaccharide as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ = 8.02-7.25 (m, 30 H), 5.73 (t, J = 9.0 Hz, 1 H), 5.72 (m, 1 H), 5.61 (t, J = 10.0 Hz, 1 H), 5.50-5.40 (m, 2 H), 5.22 (s, 1 H), 5.10 (dd, J = 10.5 Hz, 1 H), 4.79 (dd, J = 7.50 Hz, 1 H), 4.49 (d, J = 11.50 Hz,1 H), 4.41 (dd, J = 12.00, 4.5 Hz, 1 H), 4.25 (dd, J = 12.0, 4.50 Hz, 1 H), 4.12 (t, J = 9.50 Hz, 1 H), 4.05 (dd, J = 13.0, 6.5 Hz, 1 H), 3.78 (dd, J = 9.5, 2.5 Hz, 1 H), 3.64 (t, J = 9.5 Hz, 1 H), 3.61 (dd, J = 11.0, 4.50 Hz, 1 H), 3.32 (m, 1 H), 2.81 (t, J = 9.5 Hz, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$, ppm): 165.72, 165.41, 165.23, 165.18, 164.92, 136.55, 133.38, 133.35, 133.29, 133.26, 133.17, 133.07, 129.94, 129.84, 129.80, 129.77, 129.68, 129.59, 129.33, 129.25, 129.02, 128.48, 128.45, 128.34, 128.25, 128.16, 128.13, 126.05, 117.75, 101.90, 101.21, 99.36, 78.23, 73.30, 72.90, 72.45, 72.23, 72.04, 71.93, 71.72, 69.94, 67.63, 66.39, 62.32. HRMS (ESI) calcd for $C_{57}H_{54}NO_{16}$ $[M+NH_4]^+$: 1008.3443; found: 1008.3639.

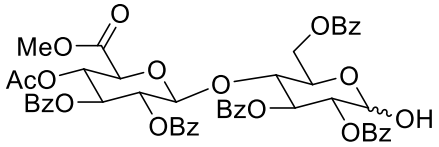


Compound 5: To a solution of **4** (180 mg, 0.182 mmol) in MeOH (20 mL) and DCM (20 mL) was added a catalytic amount of $TsOH{\cdot}H_2O$ (0.2 g). The mixture was stirred at room temperature for 4 h, and the mixture was concentrated under vacuum. The residue was dissolved in DCM (75 mL) and washed with saturated $NaHCO_3$ (30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude mixture was purified by column chromatography using 50 % EtOAc in toluene as eluent, affording **5** as colorless oil (130 mg, 81% yield). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ = 8.02-759 and

7.54-7.25 (2m, 25 H), 5.71 (m, 2 H), 5.43 (t, *J* = 8.0 Hz, 1 H), 5.36 (t, *J* = 8.0 Hz, 1 H), 5.25 (t, *J* = 9.5 Hz, 1 H), 5.13 (d, *J* = 15.0 Hz, 1 H), 5.05 (d, *J* = 10.5 Hz,1 H), 4.79 (d, *J* = 8.0 Hz, 1 H), 4.72 (d, *J* = 8.0 Hz, 1 H), 4.58 (dd, *J* = 10.5, 1.5 Hz, 1 H), 4.41 (dd, *J* = 12.0, 5.0 Hz,1 H), 4.23 (dd, *J* = 13.0, 5.0 Hz, 1 H), 4.15 (t, *J* = 9.5 Hz, 1 H), 4.05 (dd, *J* = 13.0, 6.0 Hz, 1 H), 3.80 (m, 1 H), 3.73 (t, *J* = 9.5 Hz, 1 H), 3.37 (t, *J* = 10.0 Hz, 1 H), 3.26 (dd, *J* = 12.0, 2.5 Hz, 1 H), 3.20 (dd, *J* = 12.5, 2.5 Hz, 1 H). HRMS (ESI) calcd for $C_{50}H_{47}O_{16}[M+H]^+$: 903.2879; found: 903.2848.

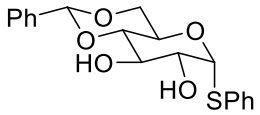


Compound 6: To a solution of **5** (100 mg, 0.111 mmol) in DCM (16 mL) was added (diacetoxy)iodobenzene (89.25 mg, 0.277 mmol), TEMPO (3.5 mg, 0.022 mmol), and water (4 mL). The mixture was stirred vigorously overnight, and TLC showed that the starting material was totally consumed (HRMS (ESI) for calcd acid: $C_{50}H_{48}NO_{17}[M+NH_4]$: 934.2922, found: 934.2992). A freshly prepared solution of diazomethane in Et_2O (50 mL) was added. The mixture was stirred for 30 min, and acetic acid (0.5 mL) was added to react with remaining diazomethane. After removal of solvent, the crude product was dissolved in DCM (10 mL), followed by addition of triethyl amine (1 mL), Ac_2O (0.3 mL) and DMAP (0.05 g). The reaction mixture was stirred at room temperature for 3 h and then quenched with methanol (0.2 mL). After dilution with DCM (20 mL), the mixture was washed with 10% aqueous HCl (20 mL) and saturated aqueous $NaHCO_3$ (30 mL) sequentially. The organic layer was concentrated and the crude product was purified by silica gel column chromatography using 40 % EtOAc in hexane as eluent, giving a clear oil (84 mg, 82 % overall yield). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ = 7.97-7.19 (m, 25 H), 5.75 (t, *J* = 9.0 Hz, 1 H), 5.70 (ddd, *J* = 16.5,11.0,5.5 Hz, 1 H), 5.57 (t, *J* = 9.5 Hz, 1 H), 5.44 (t, *J* = 8.5 Hz, 1 H), 5.36 (t, *J* = 8.0 Hz, 1 H), 5.27 (t, *J* = 9.5 Hz, 1 H), 5.13 (d, *J* = 17.0 Hz, 1 H), 5.05 (d, *J* = 10.5 Hz, 1 H), 4.95 (d, *J* = 8.0 Hz, 1 H), 4.73 (d, *J* = 7.5 Hz, 1 H), 4.63 (d, *J* = 12.0 Hz, 1 H), 4.37 (dd, *J* = 11.5, 3.5 Hz, 1 H),4.27 (t, *J* = 9.5 Hz, 1 H), 4.25 (dd, *J* = 11.0, 2.5 Hz, 1 H), 4.02 (dd, *J* = 13.0, 5.5 Hz, 1 H), 3.82 (d, *J* = 9.5 Hz, 1 H), 3.36 (s, 3 H), 1.84 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 164.34, 161.25, 160.89, 160.73, 160.47, 160.38, 159.97, 128.67, 128.58, 128.54, 128.42, 128.35, 128.31, 125.03, 125.00, 124.95, 124.94, 124.85, 124.72, 124.57, 123.81, 123.63, 123.53, 112.84, 96.23, 94.55, 68.66, 68.15, 68.01, 67.47, 67.39, 66.89, 65.11, 64.48, 57.51, 47.77, 17.75. HRMS (ESI) calcd for $C_{53}H_{52}NO_{18}[M+NH_4]^+$: 990.3184; found: 990.3118.

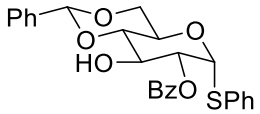


Compound 7: To a solution of compound **6** (105 mg, 0.11 mmol) in a mixture of toluene (25 mL) and absolute ethanol (10 mL) was added Wilkinson's catalyst (chlorotris(triphenyl phosphine)rhodium(I)) (40 mg, 0.043 mmol). The reaction mixture was refluxed for 5 h, and the solvent was removed by evaporation under vacuum. The crude mixture was dissolved in THF (9 mL) and water (1 mL), followed by addition of NIS (0.15 mmol). The mixture was stirred for 30 min, the solvent was removed under vacuum, and the residue was subjected to silica gel column chromatography using 25% EtOAc in

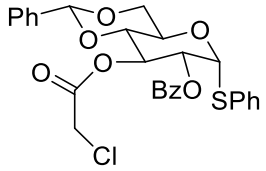
hexane affording the desired product as a white solid (84 mg, 82% yield). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ = 8.03-7.12 (m, 25 H), 6.14 (t, *J* = 10.0 Hz, 1 H), 5.61 (t, *J* = 9.5 Hz, 1 H), 5.56 (d, *J* = 3.5 Hz, 1 H), 5.46 (dd, *J* = 9.5, 8.0 Hz, 1 H), 5.27 (t, *J* = 9.5 Hz, 1 H), 5.10 (dd, *J* = 10.5, 3.5 Hz, 1 H), 5.02 (d, *J* = 7.5 Hz,1 H), 4.62 (dd, *J* = 13.0, 3.0 Hz,1 H), 4.36 (m, 2 H), 4.23 (t, *J* = 9.5 Hz, 1 H), 3.83 (d, *J* = 9.5 Hz, 1 H), 3.41 (s, 3 H), 1.84 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 163.97, 160.94, 160.72, 160.61, 160.35, 159.89, 159.61, 128.23, 128.16, 128.11, 127.96, 127.85, 124.74, 124.59, 124.50, 124.47, 124.46, 123.72, 123.36, 123.19, 123.15, 122.89, 95.83, 84.97, 67.72, 67.42, 67.33, 67.05, 66.57, 65.20, 64.12, 63.12, 56.88, 47.37. 17.75. HRMS (ESI) calcd for $C_{50}H_{48}NO_{18}[M+NH_4]^+$: 950.2871; found: 950.2707.



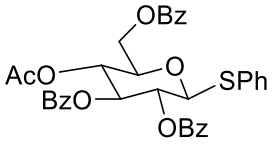
Compound 9: Compound **8** (1.94 g, 4.41 mmol) was dissolved in a mixture of methanol (20 mL), and THF (20 mL). To this mixture was added a solution of NaOMe in methanol (1 *M*, 1 mL). The reaction mixture was stirred for 3 h before AcOH (1 mL) was added. The neutralized solution was treated with amberlite (5 g), and stirred for 30 min. After filtration, the filtrate was concentrated under reduced pressure. The resulting mixture was dissolved in dry DMF (20 mL), followed by addition of benzaldehyde dimethyl acetal (2 mL) and $TsOH{\cdot}H_2O$ (0.15 g, 0.8 mmol). The reaction mixture was warmed to 70 °C. After 3 h, TLC showed that the starting material was consumed completely, and Et_3N (1 mL) was then added. After solvent was removed under vacuum, the mixture was subjected to silica gel column chromatography using 50% EtOAc in hexanes, affording a white solid (1.33 g, 84 %). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ = 7.53-7.26 (m, 10 H), 5.61 (d, *J* = 5.5 Hz, 1 H), 5.56 (s, 1 H), 4.37-4.28 (m, 2 H), 3.98 (m, 1 H), 3.87 (t, *J* = 10.5 Hz, 1 H), 3.79 (t, *J* = 10.0 Hz, 1 H), 3.56 (t, *J* = 8.5, 1 H), 2.85 (brs, 1 H), 2.60 (brs, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 136.92, 133.51, 132.18, 129.33, 129.20, 128.36, 127.92, 126.33, 102.06, 90.92, 80.97, 72.52, 72.27, 68.66, 63.94. HRMS (ESI) calcd for $C_{19}H_{20}NaO_5S$ $[M+Na]^+$: 383.0923; found: 383.1056.



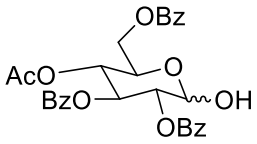
Compound 10: To a solution of compound **9** (1 g, 2.8 mmol) in pyridine (40 mL) was added drop wise benzoyl chloride (0.39 g, 0.32 mL, 2.8 mmol) at -50 °C. After 30 min, the reaction was warmed to room temperature and stirred for 1 h. After addition of methanol (0.5 mL), the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography (silica gel) using 25% EtOAc in hexanes, affording a white solid (0.97 g, 75 %). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ = 8.14-8.13 and 7.63-7.26 (2 m, 15 H), 5.99 (d, *J* = 6.0 Hz, 1 H), 5.60 (s, 1 H), 5.33 (dd, *J* = 10.0, 6.0 Hz, 1 H), 4.48 (dt, *J* = 10.0 Hz, 1 H), 4.33 (t, *J* = 10.0 Hz, 1 H), 4.28 (dd, *J* = 5.5 Hz, 1 H), 3.81 (t, *J* = 10.0 Hz, 1 H), 3.70 (t, *J* = 9.50 Hz, 1 H), 2.72 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 165.86, 136.92, 133.54, 132.91, 132.35, 130.04, 129.39, 129.34, 129.10, 128.53, 128.40, 127.81, 126.36, 102.12, 86.48, 81.24, 73.68, 69.52, 68.60, 63.21. HRMS (ESI) calcd for $C_{26}H_{25}O_6S$ $[M+H]^+$: 465.1366, found: 465.1345.



Compound 11: To a solution of **10** (500 mg, 1.01 mmol) in DCM (20 mL) and pyridine (1 mL) was added chloroacetic anhydride (183 mg, 1.11 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then warmed to room temperature. After another 2 h, the reaction mixture was diluted with DCM (30 mL) and washed with 5% aqueous HCl (200 mL × 2). The organic phase was concentrated and then purified by silica gel chromatography using 40% EtOAc in hexanes, affording a white solid (502 mg, 92% yield). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ = 8.09-7.26 (m, 15 H), 6.065 (d, *J* = 5.50 Hz, 1 H), 5.83 (t, *J* = 10.0 Hz, 1 H), 5.58 (s, 1 H), 5.39 (dd, *J* = 9.5, 6.0 Hz, 1 H), 4.58 (td, *J* = 10.0 Hz, 1 H), 4.30 (dd, *J* = 11.0, 5.25 Hz, 1 H), 4.03 (q, *J* = 15.29 Hz, 2 H), 3.86 (t, *J* = 10.0 Hz, 1H), 3.84 (t, *J* = 10.5 Hz, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 166.46, 165.49, 136.70, 133.82, 132.48, 132.39, 130.10, 129.26, 129.17, 128.68, 128.33, 127.98, 126.23, 101.77, 86.41, 78.71, 71.95, 71.21, 68.51, 63.61, 40.54. HRMS (ESI) calcd for $C_{28}H_{25}ClNaO_7S$ $[M+Na]^+$: 563.0901, found: 563.0909.

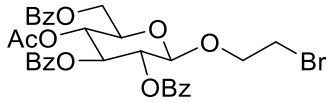


Compound 13: To a solution of **12** (960 mg, 2.04 mmol) in DCM (30 mL) and pyridine (7 mL) was added benzoyl chloride (342 mg, 2.44 mmol, 1.2 eq) at -78 °C drop wise. The reaction mixture was allowed to stirr for 1 h at that temperature and then warmed to room temperature. Acetic anhydride (2 mL) was added. After another 5 h, the reaction mixture was diluted with DCM (30 mL) and washed with 5% aqueous HCl (200 mL × 2) and saturated $NaHCO_3$ (50 mL). The organic phase was concentrated and purified by silica gel chromatography using 20% EtOAc in hexane, affording a clear oil (830 mg, 65 %). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ = 8.11-7.14 (m, 20 H), 5.74 (t, *J* = 9.5 Hz, 1 H), 5.45 (t, *J* = 10.0 Hz, 1 H), 5.39 (t, *J* = 9.5 Hz, 1 H), 5.02 (d, *J* = 9.5 Hz, 1 H), 4.66 (dd, *J* = 12.5, 2.0 Hz, 1 H), 4.47 (dd, *J* = 12.0, 5.5 Hz, 1 H), 4.09 (m, 1 H), 1.95 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 169.43, 166.12, 165.74, 165.04, 133.44, 133.36, 133.34, 133.19, 131.65, 129.88, 129.86, 129.83, 129.62, 129.17, 128.89, 128.75, 128.51, 128.45, 128.43, 128.34, 86.07, 76.12, 74.39, 70.33, 68.58, 62.84, 20.55. HRMS (ESI) calcd for $C_{35}H_{31}O_9S$ $[M+H]^+$: 627.1683, found: 627.1720.

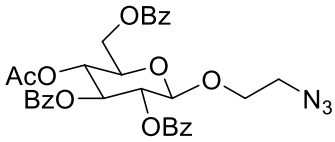


Compound 14: To a solution of compound **13** (800 mg, 1.28 mmol) in a mixture of acetone (40 mL) and water (8 mL) was added NBS (450 mg, 2.56 mmol) in small portions. The reaction mixture was stirred vigorously for 2 h. A saturated solution of sodium sulfite (100 mL) was added to the mixture, and the mixture was stirred for another 1 h. After dilution with DCM (200 mL), the organic phase was separated, and the aqueous phase was extracted with DCM (100 mL × 2). The combined organic phase was concentrated under vacuum. The resulting crude mixture was purified by flash column chromatography

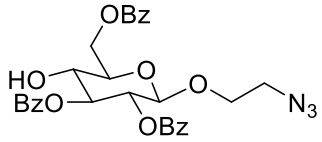
(silica gel) using 25% EtOAc in hexanes affording a white solid (581 mg, 85 % yield, alpha/beta = 8/1). ^{1}H NMR (500 MHz, $CDCl_3$, ppm, alpha isomer): δ = 8.08 (m, 15 H), 6.08 (t, *J* = 6.5 Hz, 1 H), 5.72 (d, *J* = 3.0, 1 H), 5.49 (t, *J* = 4.5 Hz, 1 H), 5.24 (dd, *J* = 10.5, 3.5 Hz,1 H), 4.58 (dd, *J* = 12.5, 2.5 Hz, 1 H), 4.52 (td, *J* = 10.0, 3.5 Hz, 1 H), 4.40 (dd, *J* = 12.0, 4.0 Hz, 1 H), 1.93 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 169.65, 166.50, 166.01, 165.90, 133.42, 133.32, 133.25, 129.91, 129.82, 129.78, 129.75, 129.62, 129.17, 128.97, 128.46, 128.43, 90.38, 72.28, 70.52, 68.72, 67.47, 62.61, 20.57. HRMS (ESI) calcd for $C_{29}H_{30}NO_{10}$ $[M+NH_4]^+$: 552.1864, found: 552.1849.



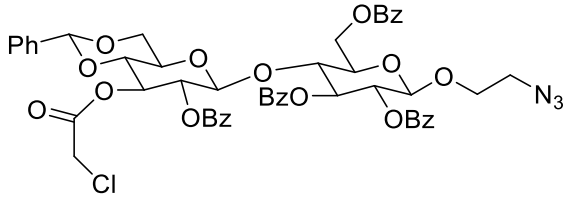
Compound 15: Compound **14** (500 mg, 0.936 mmol) was dissolved in DCM (30 mL). Dry potassium carbonate (1.2 g) was added, followed by addition of excess trichloroacetonitrile (592 µL, 5.82 mmol). The reaction was stirred for 8 h at room temperature. Solids were removed by filtration, and the filtrate was concentrated under vacuum. The resulting donor was then mixed with 2-bromoethanol (125 mg, 1 mmol) and 4 Å molecular sieves (600 mg). The reaction mixture was stirred for 1 h in DCM (7 mL, then TMSOTf (16 µL, 0.0845 mmol) was added. The reaction mixture was allowed to stir for another 12 h and then quenched with triethyl amine (0.5 mL). After removal of molecular sieves by filtration, the filtrate was concentrated, and the product was purified by silica gel column chromatography (EtOAc/hexane: 1/4), affording compound **15** as a clear oil (533 mg, 89% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.10-7.35 (m, 15 H), 5.72 (t, *J* = 9.0 Hz, 1 H), 5.478 (dd, *J* = 9.0, 7.5 Hz, 1 H), 5.44 (t, *J* = 9.5 Hz, 1 H), 4.88 (d, *J* = 8.0 Hz, 1 H), 4.62 (dd, *J* = 12.25, 7.5 Hz, 1 H), 4.47 (dd, *J* = 12.25, 5.0 Hz, 1 H), 4.16 (m, 1 H), 4.05 (ddd, *J* = 9.5, 4.5, 2.0 Hz, 1 H), 3.88 (td, *J* = 11.5, 7.0 Hz, 1 H), 3.42 (m, 2 H), 1.94 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 169.45, 166.22, 165.84, 165.16, 133.48, 133.36, 165.16, 133.48, 133.36, 133.28, 129.83, 129.80, 129.53, 129.20, 128.73, 128.55, 128.48, 128.36, 101.34, 72.98, 72.17, 71.53, 69.82, 68.75, 62.62, 29.58, 20.57. HRMS (ESI) calcd for $C_{31}H_{33}BrNO10$ $[M+NH_4]^+$: 658.1282, found: 658.1296.



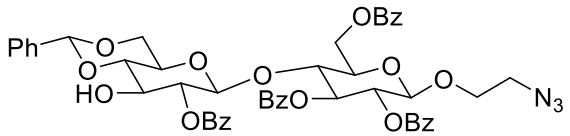
Compound 16: To a solution of compound **15** (533 mg, 0.833 mmol) in DMF (20 mL) was added NaN_3 (542 mg, 8.33 mmol). The reaction was warmed to 60 °C and stirred overnight. After removal of solids via filtration, the filtrate was concentrated under vacuum. The crude product was purified by silica gel column chromatography using 40 % EtOAc in hexanes, giving a clear oil (487 mg, 97% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.11-7.27 (m, 15 H), 5.73 (t, *J* = 9.5 Hz, 1 H), 5.50 (dd, *J* = 10.0, 8.0 Hz, 1 H), 5.46 (t, *J* = 9.5 Hz, 1 H), 4.88 (d, *J* = 8.5 Hz, 1 H), 4.63 (dd, *J* = 12.5, 3.5 Hz, 1 H), 4.48 (dd, *J* = 12.0, 5.0 Hz, 1 H), 4.04 (m, 2 H), 3.73 (ddd, *J* = 11.5, 8.0, 4.0 Hz, 1 H), 3.42 (ddd, *J* = 12.0, 8.0, 4.0 Hz, 1 H), 3.29 (ddd, *J* = 13.5, 5.0, 4.0 Hz, 1 H), 1.94 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 169.36, 166.17, 165.81, 165.07, 133.41, 133.31, 133.19, 129.82, 129.78, 129.62, 129.29, 128.80, 128.51, 128.44, 128.31, 101.15, 73.09, 72.21, 71.59, 68.79, 68.38, 62.63, 50.60, 20.53. HRMS (ESI) calcd for $C_{31}H_{33}N4O10$ $[M+NH_4]^+$: 621.2191, found: 621.2242.



Compound 17: To a solution of compound **16** (487 mg, 0.808 mmol) in a mixture of THF (20 mL) and MeOH (10 mL) was added AcCl (0.5 mL) at 0 °C. The mixture was stirred at room temperature for 10 h, followed by addition of Et_3N (1 mL). After removal of solvents, the residue was subjected to silica gel column chromatography using 50% EtOAc in hexanes, affording a clear oil (354 mg, 78% yield). ^1H NMR ($CDCl_3$, 500 MHz): δ = 8.09-7.26 (m, 15 H), 5.52 (t, *J* = 9.5 Hz, 1 H), 5.47 (dd, *J* = 10.0, 8.0 Hz, 1 H), 4.84 (d, *J* = 8.0 Hz, 1 H), 4.76 (dd, *J* = 12.5, 4.5 Hz, 1 H), 4.70 (dd, *J* = 12.0, 2.5 Hz, 1 H), 4.035 (ddd, *J* = 10.5, 5.0, 4.0 Hz, 1 H), 3.94 (t, *J* = 9.5 Hz, 1 H), 3.866 (ddd, *J* = 10.0, 4.5, 2.5 Hz, 1 H), 3.73 (ddd, *J* = 11.5, 8.0, 4.0 Hz, 1 H), 3.42 (ddd, *J* = 11.5, 8.0, 4.0 Hz, 1 H), 3.29 (td, *J* = 13.0, 4.5 Hz, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 167.26, 166.92, 165.28, 133.50, 133.35, 133.18, 129.96, 129.84, 129.75, 129.60, 129.37, 128.90, 128.48, 128.41, 128.32, 101.10, 76.47, 74.61, 71.35, 69.50, 68.36, 63.34, 60.41, 50.63. HRMS (ESI) calcd for $C_{29}H_{28}N_3O_9$ $[M+NH_4]^+$: 562.1820, found: 562.1807.

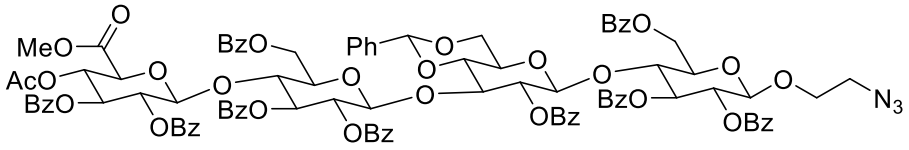


Compound 18: A mixture of compound **11** (85 mg, 0.175 mmol), compound **17** (115 mg, 0.225 mmol), 3 Å molecular sieves (300 mg) and DCM (6 mL) was stirred at room temperature for 1 h and then cooled to -40 °C. NIS (42.2 mg, 0.188 mmol) was subsequently added, followed by addition of TMSOTf (4 µL, 0.0157 mmol). The reaction mixture was allowed to warm to room temperature. After 1 h, the reaction was quenched with Et_3N (0.1 mL). Filtration and concentration gave the crude product, which was purified by silica gel column chromatography using 25% EtOAcs in hexane to give the desired product as a white solid (98 mg, 57% yield). ^1H NMR ($CDCl_3$, 500 MHz): δ = 8.05-7.31 (m, 25 H), 5.70 (t, *J* = 9.0 Hz, 1 H), 5.40 (dd, *J* = 9.5, 2.0 Hz,1 H), 5.8 (d, *J* = 8.8 Hz, 1 H), 5.28 (dd, *J* = 9.5, 8.0 Hz, 1 H), 5.20 (s, 1 H), 4.78 (d, *J* = 7.5, 1 H), 4.75 (d, *J* = 7.5 Hz, 1 H), 4.51 (dd, *J* = 12.0, 1.5 Hz, 1 H), 4.38 (dd, *J* = 12.0, 4.5 Hz, 1 H), 4.107 (t, *J* = 9.5 Hz, 1 H), 3.90 (ddd, *J* = 8.5, 1.5, 0.5 Hz, 1H), 3.90 (d, *J* = 6.5 Hz, 2 H), 3.79 (m, 1 H), 3.63 (ddd, *J* = 10.5, 8.0, 4.0 Hz, 1 H), 3.58 (dd, *J* = 10.5, 5.0, 1 H), 3.53 (t, *J* = 9.5 Hz, 1 H), 3.34 (ddd, *J* = 10.5, 7.5, 3.5 Hz, 1 H), 3.26-3.20 (m, 2 H), 2.78 (t, *J* = 10.5 Hz, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 165.43, 164.70, 164.17, 164.09, 163.98, 135.37, 132.62, 132.39, 132.30, 132.15, 128.97, 128.84, 128.78, 128.74, 128.64, 128.50, 128.26, 128.20, 127.63, 127.60, 127.46, 127.29, 127.21, 125.19, 125.06, 100.68, 100.32, 99.74, 76.60, 72.30, 72.18, 71.95, 71.36, 70.55, 67.35, 66.54, 65.17, 61.07, 49.52, 39.35, 28.68. HRMS (ESI) calcd for $C_{51}H_{47}ClN_3O_{16}$ $[M+H]^+$: 992.2645, found: 992.2471.

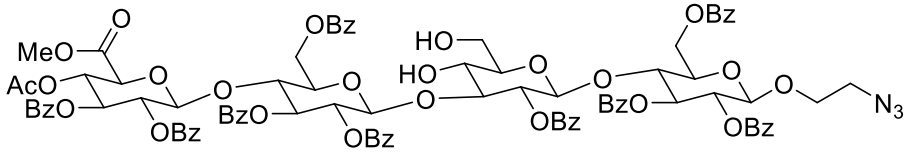


Compound 19: To a solution of compound **18** (90 mg, 0.091 mmol) in a mixture of

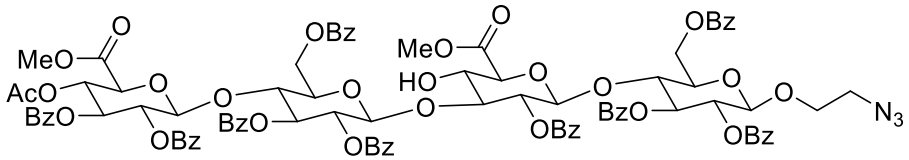
toluene (16 mL) and ethanol (16 ml) was added DABCO (1.32 g, 1.32 mmol, 14.5 eq). The reaction mixture was warmed to 60 °C and stirred for 3 h. The reaction mixture was washed with 5% aqueous HCl (500 mL), saturated $NaHCO_3$ (100 mL), and dried over Na_2SO_4. After removal of the solvents, the crude mixture was subjected to silica gel column chromatography using 50% EtOAc in hexanes, affording a white solid (69 mg, 83 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.09-7.92 and 7.64-7.26 (m, 25 H), 5.69 (t, *J* = 9.5 Hz, 1 H), 5.41 (dd, *J* = 9.5, 8.0 Hz, 1 H), 5.23 (s, 1 H), 5.18 (dd, *J* = 9.0, 8.0 Hz, 1 H), 4.73 (d, *J* = 8.0 Hz, 1 H), 4.70 (d, *J* = 7.5 Hz, 1 H), 4.50 (m, 2 H), 4.09 (t, *J* = 9.5 Hz, 1 H), 3.91 (m, 1 H), 3.89 (t, *J* = 9.5 Hz, 1 H), 3.80 (td, *J* = 10.0, 3.5 Hz, 1 H), 3.63 (ddd, *J* = 10.5, 8.0, 4.0 Hz, 1 H), 3.58 (dd, J = 10.5, 5.0, 1H), 3.37 (t, J = 9.0 Hz, 1H), 3.34 (ddd, J = 10.5, 7.5, 3.5 Hz, 1H), 3.22 (ddd, *J* = 13.0, 5.5, 4.0 Hz, 1 H), 3.15 (ddd, *J* = 14.0, 10.0, 5.0 Hz, 1 H), 2.71 (t, *J* = 10.5 Hz, 1 H), 2.69 (brs, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 165.90, 165.51, 165.22, 165.12, 136.66, 133.43, 133.34, 133.28, 133.15, 129.92, 129.87, 129.79, 129.78, 129.65, 129.58, 129.31, 128.88, 128.52, 128.50, 128.40, 128.29, 126.19, 101.65, 100.81, 80.32, 74.62, 73.13, 73.03, 72.36, 71.53, 68.37, 67.60, 66.04, 62.37, 50.53. HRMS (ESI) calcd for $C_{49}H_{49}N_4O_{15}$ $[M+NH4]^+$: 933.3194, found: 933.3019.



Compound 20: A mixture of compound **7** (55 mg, 0.059 mmol), dry potassium carbonate (300 mg), DCM (10 mL) and trichloroacetonitrile (3 mL) was stirred at room temperature for 5 h. The solid was removed by filtration through a celite pad. After the filtrate was concentrated, the product was purified by flash chromatography using silica gel column, with 50% of EtOAc in hexane as eluent, to give a white solid. This solid was mixed with compound **19** (40 mg, 0.044 mmol), 4 Å molecular sieves (300 mg) and DCM (6 mL). After stirring at room temperature for 1 h, TMSOTf (6 µL) was added. The reaction was allowed to stirr for another 7 h, followed by addition of Et_3N (0.1 mL). After filtration and concentration, the crude product was purified by silica gel column chromatography using 50% EtOAc in hexane as eluent, affording tetrasaccharide **20** as a white solid (62 mg, 78% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.96-7.07 (m, 50 H), 5.59 (t, *J* = 9.0 Hz, 1 H), 5.44 (dt, *J* = 7.5, 2.0 Hz, 2 H), 5.32 (dd, *J* = 9.5, 8.0 Hz, 1 H), 5.29 (dd, *J* = 9.5, 8.0 Hz, 1 H), 5.21-5.12 (m, 3 H), 4.75 (d, *J* = 7.5 Hz, 1 H), 4.68 (d, *J* = 8.0 Hz, 1 H), 4.65 (d, *J* = 7.5 Hz, 1 H), 4.54 (d, *J* = 8.0 Hz, 1 H), 4.30 (t, *J* = 11.0 Hz, 1 H), 4.27 (s, 1 H), 4.13 (t, *J* = 9.5 Hz, 1 H), 4.02 (dd, *J* = 9.0, 4.5 Hz, 1 H), 3.96 (q, *J* = .5 Hz, 1 H), 3.84 (ddd, *J* = 11.0, 6.5, 5.5 Hz, 1 H), 3.62 (d, *J* = 10.0 Hz, 1 H), 3.59 (m, 2 H), 3.52 (d, *J* = 10.0 Hz, 1 H), 3.51 (dd, *J* = 12.0, 5.0 Hz, 1 H), 3.40 (m, 1 H), 3.32 (m, 1 H), 3.28 (s, 3 H), 3.17 (ddd, *J* = 13.5, 5.0, 4.0 Hz, 1 H), 3.10 (ddd, *J* = 9.5, 6.0, 5.0 Hz, 1 H), 2.70 (t, *J* = 10.5 Hz, 1 H), 1.85 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 165.08, 161.91, 161.76, 161.50, 161.23, 161.01, 160.79, 160.62, 160.14, 132.65, 129.42, 129.35, 129.27, 129.22, 129.16, 129.02, 128.93, 128.61, 125.87, 125.81, 125.64, 125.59, 125.47, 125.33, 124.96, 124.61, 124.47, 124.42, 124.32, 124.25, 123.99, 121.91, 97.65, 97.55, 96.82, 96.73, 95.90, 75.21, 71.96, 69.63, 69.07, 69.00, 68.91, 68.78, 68.47, 68.29, 67.54, 65.17, 64.34, 63.71, 62.22, 58.17, 58.05, 48.48, 46.54, 17.71. HRMS (ESI) calcd for $C_{99}H_{91}N_4O_{32}$ $[M+NH4]^+$: 1848.5650, found: 1848.5625.

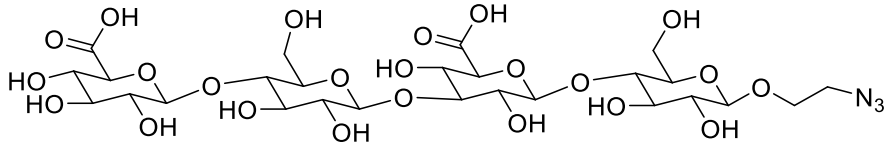


Compound 21: A mixture of compound **20** (50 mg, 0.0273 mmol), $TsOH{\cdot}H_2O$ (0.02 g), DCM (10 mL) and MeOH (4 mL) was stirred at room temperature for 8 h. Et_3N (0.1 mL) was added, and the solvent was removed under vacuum. The resulting mixture was subjected to column chromatography using 50% EtOAc in hexane as eluent, affording the diol as a white solid (42 mg, 88% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.94-7.04 (m, 45 H), 5.59 (q, *J* = 9.5 Hz, 2 H), 5.37 (p, *J* = 8.5 Hz, 2 H), 5.23 (t, *J* = 9.5 Hz, 2 H), 5.05 (t, *J* = 8.5 Hz,1 H), 4.88 (d, *J* = 8.0 Hz, 1 H), 4.62 (t, *J* = 8.0 Hz, 2 H), 4.46 (d, *J* = 8.0 Hz,1 H), 4.35 (d, *J* = 11.0 Hz, 1 H), 4.28 (dd, *J* = 10.0, 5.0 Hz,1 H), 4.20 (dd, *J* = 12.0, 4.5 Hz, 1 H), 4.07 (t, *J* = 9.5 Hz, 1 H), 4.01 (t, *J* = 9.5 Hz, 1 H), 3.85 (m, 1 H), 3.81 (d, *J* = 10.0 Hz, 1 H), 3.66 (s, 1 H), 3.61-3.55 (m, 2 H), 3.36-3.27 (m, 4 H), 3.28 (s, 3 H), 3.17 (ddd, *J* = 13.5, 6.0, 5.0 Hz, 1 H), 3.03-2.90 (m, 1 H).1.82 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 169.07, 165.86, 165.81, 165.67, 165.46, 165.16, 165.03, 164.92, 164.68, 163.90, 133.44, 133.39, 133.30, 133.13, 133.04, 132.94, 132.57, 129.80, 129.79, 129.74, 129.71, 129.67, 129.59, 129.55, 129.44, 129.32, 129.21, 129.02, 128.68, 128.57, 128.49, 128.42, 128.39, 128.30, 128.27, 128.20, 127.91, 127.78, 101.29, 100.90, 100.87, 100.75, 85.29, 76.38, 75.82, 75.63, 73.16, 73.09, 72.97, 72.85, 72.59, 72.09, 71.99, 71.54, 71.49, 71.37, 69.13, 69.02, 68.27, 62.20, 62.16, 61.67, 52.46, 50.47, 29.69, 20.30. HRMS (ESI) calcd for $C_{92}H_{87}N_4O_{32}$ $[M+NH_4]^+$: 1759.5303, found: 1759.5351.

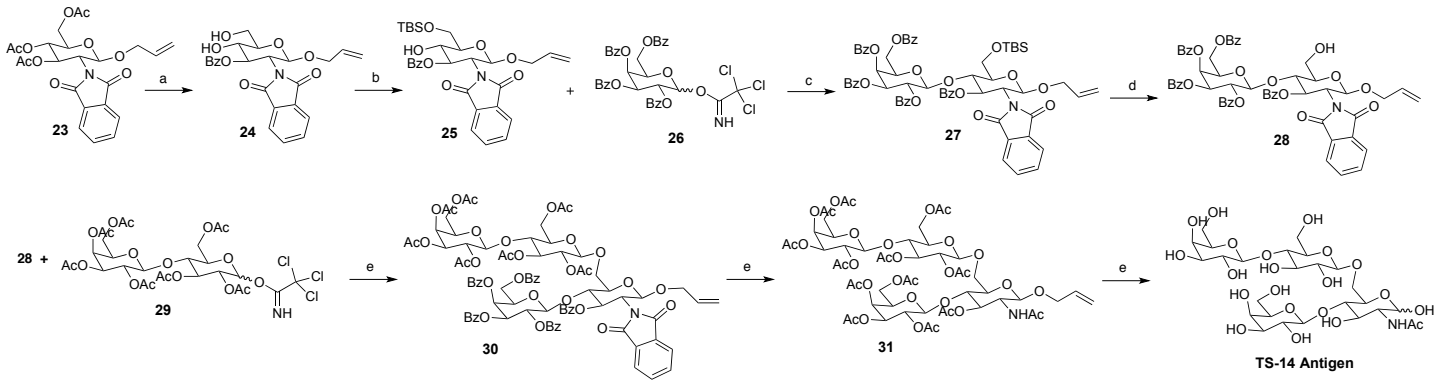


Compound 22: A mixture of compound **21** (36 mg, 0.02 mmol), diacetoxyiodobenzene (BAIB, 16.6 mg, 0.05 mmol), TEMPO (0.93 mg, 0.006 mmol), DCM (6 ml), and water (1.5 mL) was stirred at room temperature for 40 h. After dilution with DCM (20 mL), the resulting mixture was washed with water (20 mL), concentrated under vacuum and diluted with DCM (5 mL). To this solution was added freshly prepared diazamethane in ether (30 mL), and the reaction mixture was stirred for 1 h. After addition of 0.2 mL of AcOH to the mixture, the solvent was removed under vacuum. The crude product was subjected to column chromatography using 50% EtOAc in hexanes as eluent, affording a white solid (26 mg, 73% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.93-7.05 (m, 45 H), 5.59 (t, *J* = 9.50 Hz, 1 H), 5.57 (t, *J* = 9.5Hz, 1 H), 5.56 (t, *J* = 9.50 Hz, 1 H), 4.38 (t, *J* = 8.0 Hz, 1 H), 5.27 (t, *J* = 8.0 Hz, 1 H), 5.23 (t, *J* = 9.5 Hz, 1 H), 5.14 (t, *J* = 8.0 Hz, 1 H), 4.87 (d, *J* = 8.0 Hz, 1 H), 4.62 (t, *J* = 8.0 Hz, 1 H), 4.61 (d, *J* = 8.5 Hz, 1 H), 4.58 (d, *J* = 8.5 Hz, 1 H), 4.42 (d, *J* = 11.5 Hz,1 H), 4.26 (dd, *J* = 8.5, 5.5 Hz, 1 H), 4.20 (dd, *J* = 12.0, 4.0 Hz, 1 H), 4.10 (t, *J* = 10.5 Hz, 1 H), 4.08 (t, *J* = 10.0 Hz, 1 H), 3.84-3.76 (m, 4 H), 3.65 (t, *J* = 9.0 Hz, 1 H), 3.60-3.53 (m, 3 H), 3.35 (m, 1 H), 3.25 (m, 1 H), 3.34 (s, 3 H), 3.27 (s, 3 H), 3.16 (td, *J* = 13.0, 4.5 Hz, 1 H), 1.82 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 165.13, 162.99, 161.92, 161.75, 161.51, 161.20, 160.99, 160.74, 159.90, 129.49, 129.36, 129.30, 129.10, 128.86, 128.63, 125.92, 125.85, 125.66, 125.62, 125.53, 125.37, 125.32, 125.16, 125.09, 124.69, 124.60, 124.48, 124.36, 124.32, 124.27, 123.97, 97.34, 97.12, 96.89, 96.62, 80.73, 72.35, 71.64, 71.29, 69.29, 68.94, 68.66, 68.19, 67.89, 67.69, 67.62, 67.56, 65.89,

65.19, 64.23, 58.03, 48.51, 48.25, 46.52, 25.75. HRMS (ESI) calcd for $C_{93}H_{87}N_4O_{33}$ $[M+NH_4]^+$: 1787.5253, found: 1787.5198.

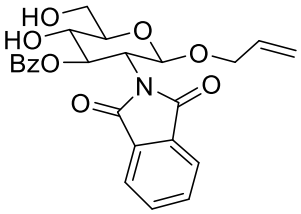


TS-3-N_3 Antigen: To a solution of compound **22** (12 mg, 0.0068 mmol) in THF (4 mL), MeOH (4 mL) was added NaOMe (0.1 mL, 1 *M* in methanol). The mixture was stirred at room temperature for 10 h before water (0.1 mL) was added. The mixture was stirred for 10 h, followed by addition of AcOH (0.1 mL). The solvent was removed under vacuum, and the resulting material was subjected to column chromatography using a mixture of EtOAc/MeOH/H_2O (60/25/20), affording desired product as a white solid (3.3 mg, 64 %). ^{1}H NMR (CD_3OD, 500 MHz): δ = 4.67 (d, *J* = 7.5 Hz, 1 H), 4.384 (d, *J* = 8.0 Hz, 1 H), 4.381 (d, *J* = 8.0 Hz, 1 H), 4.35 (d, *J* = 8.0 Hz, 1 H), 3.90 (t, *J* = 4.5 H, 1 H), 3.89 (dd, *J* = 6.0, 4.5 Hz, 1 H), 3.83 (brs, 1 H), 3.80 (brs, 1 H), 3.71-3.60 (m, 6 H), 3.54-3.33 (m, 11 H), 3.22 (t, *J* = 8.5 Hz, 1 H), 3.20 (t, *J* = 8.5 Hz, 1 H), 3.18 (t, *J* = 8.5 Hz, 1 H). ^{13}C NMR (125 MHz, CD_3OD, ppm): 175.48, 175.25, 102.30, 102.25, 102.55 (2C), 82.71, 78.91, 78.83, 75.71, 75.67, 75.24, 74.79, 74.75, 74.24, 74.11, 73.12, 72.99, 72.90, 72.73, 71.68, 70.11, 68.44, 60.00, 50.47. HRMS (ESI) calcd for $C_{26}H_{41}NaN_3O_{23}$ $[M+Na]^+$: 786.2029, found: 786.2014.



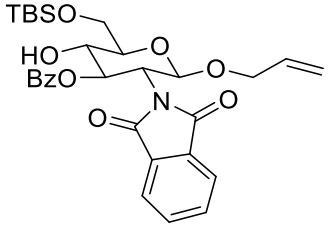
Reagents: a: i) NaOMe, MeOH, amberlite; ii) DMF, $TsOH{\cdot}H_2O$, dimethoxybenzaldehyde; iii) BzCl, DMAP, DCM, Et_3N; iiii) DCM, MeOH, $TsOH{\cdot}H_2O$, 62% yield for 4 steps. b: DCM, TBSCl, imidazole, 92% yield. c: DCM, 4 Å molecular sieves, TMSOTf, 79% yield. d: DCM, acetonitrile, HF (48%), 85% yield. e: DCM, 4 Å molecular sieves, TMSOTf, 81% yield. f: i) MeOH, NaOMe; ii) MeOH, $NH_2CH_2CH_2NH_2$; iii) Ac_2O, pyridine, DMAP, 64% yield for 3 steps. g: i) toluene, ethanol, chlorotris(triphenylphosphine)rhodium(I); ii) THF, MeOH, NaOMe, 40% yield for 2 steps.

Scheme 2. Synthesis of TS-14 Antigen

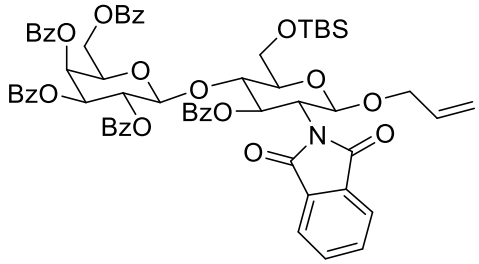


Compound 24: To a solution of compound **23** (3.33 g, 7 mmol) in methanol (80 mL) was added NaOMe (1.2 mL, 1 *M* in methanol). The mixture was stirred for 2 h. Amberlite (11 g) was added to quench the reaction. The mixture was stirred for additional 30 min then the resin was removed via filtration. The filtrate was concentrated under vacuum. The crude compound was then dissolved in DMF (30 mL), followed by addition of dimethoxybenzaldehyde (2.22 ml, 14.8 mmol) and $TsOH{\cdot}H_2O$ (0.1 g, 0.5 mmol). The mixture was stirred at 65 °C for 2 h, then Et_3N (1 mL) was added. The solvent was removed under reduced pressure, and the residue was dissolved in DCM (250 mL) and washed with water (100 mL). The organic phase was concentrated under vacuum. The resulting compound was dissolved in dry Et_3N (4 ml) and DCM (100 mL) and treated with BzCl (3.5 mL, 22.2 mmol), DMAP (0.1 g, 0.8 mmol) at 0 °C for 10 h. The reaction was quenched by addition of methanol (10 mL) at the same temperature, diluted with DCM (200 mL), washed with 0.5 N HCl (300 mL) and saturated $NaHCO_3$ solution (400 mL). The organic phase was concentrated then dissolved in DCM (30 mL) and MeOH (20 ml). $TsOH{\cdot}H_2O$ (0.2 g) was added, and the mixture was stirred at room temperature for 5 h. Et_3N (0.5 mL) was then added. The reaction mixture was then concentrated, diluted with DCM (100 mL), washed with saturated $NaHCO_3$ solution. The organic phase was concentrated and subjected to flash column chromatography using a mixture of EtOAc and hexanes (1/2 to 1/1) as eluent, giving 1.97 g (62% yield) of compound **24** as a clear oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.85-7.26 (m, 9 H), 5.926 (dd, *J* = 10.5, 8.5 Hz, 1 H), 5.72 (ddd, *J* = 21.5,11.0, 6.5 Hz, 1 H), 5.50 (d, *J* = 8.5 Hz, 1 H), 5.14 (dd, *J* = 17.5, 1.5 Hz, 1 H), 5.05 (dd, *J* = 10.0, 1.0 Hz, 1 H), 4.45 (dd, *J* = 11.0, 9.0 Hz, 1 H), 4.29 (dd, *J* = 12.0, 5.0 Hz, 1 H), 4.09 (dd, *J* = 1.5, 5.5 Hz, 1 H), 4.02 (dd, *J* = 11.5, 3.0 Hz, 1 H), 3.96 (t, *J* = 9.0 Hz, 1 H), 3.94 (dd, *J* = 12.0, 5.0 Hz, 1 H), 3.726 (td, *J* = 9.5, 4.0 Hz, 1 H), 2.61 (brs, 1 H, OH). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 167.03, 134.19, 133.49, 133.45, 131.37,

129.87, 128.83, 128.40, 123.55, 117.69, 97.32, 75.68, 74.53, 70.56, 70.31, 62.30, 54.57. HRESI-MS: $C_{24}H_{23}NO_8$ (453.1424). $[M+Na]^+$ cald: 476.1321, found: 476.1308.

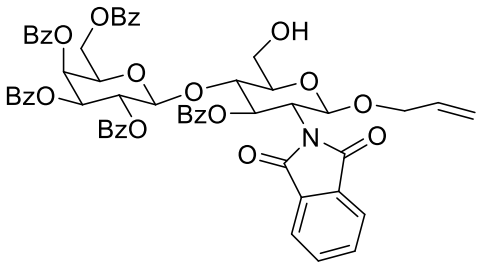


Compound 25: Compound **24** (906 mg, 2 mmol) was dissolved in DCM (30 mL), followed by addition of TBSCl (0.375 g, 2.5 mmol) and imidazole (0.286 g, 4.2 mmol). The mixture was stirred at 0 °C for 5 h. MeOH (2 mL) was added, and the mixture was washed with 5% aqueous HCl (100 mL). The organic phase was concentrated, and the product was purified by column chromatography (silica gel) using EtOAc and hexane (1/1) as eluent, affording compound **25** (1.4 g, 92% yield). ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.90-7.26, 5.93 (dd, J = 15.5, 8.5 Hz, 1 H), 5.74 (ddd, J = 22.5, 11.0, 5.5 Hz, 1 H), 5.47 (d, J = 8.0 Hz, 1 H), 5.14 (dd, J = 16.5, 1.5 Hz,1 H), 5.06 (dd, J = 10.0, 1.5 Hz, 1 H), 4.43 (dd, J = 10.5, 8.0 Hz, 1 H), 4.29 (tdd, J = 12.5, 4.5, 1.5 Hz, 1 H), 4.08 (tdd, J = 12.5, 4.5, 1.5 Hz, 1 H), 4.025 (dd, J = 10.5, 5.5 Hz, 1 H), 3.998 (dd, J = 10.5, 5.5 Hz, 1 H), 3.93 (dt, J = 9.5, 2.5 Hz, 1 H), 3.70 (td, J = 9.5, 5.5 Hz, 1 H), 3.54 (brs, 1 H, OH), 0.92 (s, 9 H), 0.139 (s, 3 H), 0.132 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 166.71, 134.07, 133.54, 133.28, 131.45, 129.89, 129.07, 128.33, 123.48, 117.60, 97.03, 74.71, 74.26, 72.59, 69.94, 64.45, 54.47, 25.00, 18.32, -5.388, -5.410. HRESI-MS: $C_{30}H_{37}NO_8Si$ (567.2288). $[M+NH4]^+$ cald: 585.2626, found: 585.2613.

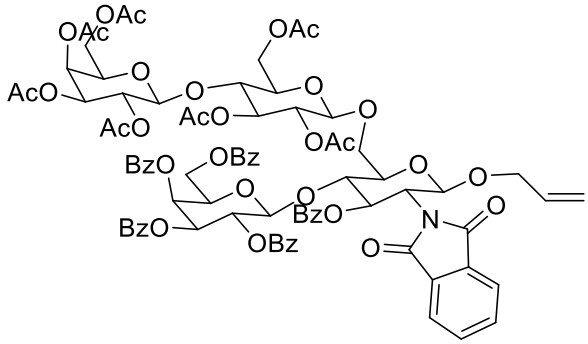


Compound 27: Compound **26** (0.45 g, 0.604 mmol), freshly prepared by treatment of 2,3,4,6-tetra-*O*-benzoyl-galacopyrannoside with trichloroacetonitrile in the presence of potassium carbonate in DCM) and acceptor **25** (0.285 g, 0.503 mmol) were mixed with molecular sieves (4 Å, 500 mg) in DCM (6 mL). The mixture was stirred at room temperature for 1 h, cooled to 0 °C, then TMSOTf (30 μL) was added. The mixture was allowed to warm to room temperature overnight. After Et_3N (0.1 mL) was added, solids were removed via filtration through a celite pad. The filtrate was concentration, and the residue was purified by flash column chromatography (silica gel) using a mixture of EtOAc and hexane (1/4 to 1/2) as eluent, affording compound **27** (0.455 g, 79% yield) as a white power. ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.06-7.09 (m, 29 H), 6.12 (dd, J = 11.0, 9.5 Hz, 1 H), 5.76 (d, J = 3.5 Hz, 1 H), 5.72 (dt, J = 11.0, 5.0 Hz, 1 H), 5.66 (dd, J = 10.5, 8.5 Hz, 1 H), 5.41 (dd, J = 10.5, 4.0 Hz, 1 H), 5.39 (d, J = 8.5 Hz, 1 H), 5.124 (d, J = 8.0 Hz, 1 H), 5.10 (dd, J = 17.0, 1.5 Hz, 1 H), 5.03 (dd, J = 11.5, 2.0 Hz, 1 H), 4.414 (dd, J = 10.5, 3.0 Hz, 1 H), 4.23 (t, J = 10.0 Hz,1 H), 4.20 (dd, J = 12.5, 4.5 Hz, 1 H), 4.01 (dd, J = 13.5, 6.5 Hz, 1 H), 3.98 (dd, J = 11.5, 11.0 Hz, 1 H), 3.91 (dd, J = 11.0, 5.5 Hz, 1 H), 3.86 (dd, J = 11.5, 2.5 Hz, 1 H), 3.78 (d, J = 10.5 Hz, 1 H), 3.61 (dd, J = 11.0, 7.5 Hz, 1 H), 3.51 (d, J =

10.0 Hz, 1 H), 0.92 (s, 9 H), 0.11 (s, 3 H), 0.08 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 163.15, 162.97, 162.92, 162.71, 162.02, 131.11, 130.86, 130.75, 130.65, 130.36, 127.39, 127.35, 127.26, 127.21, 127.17, 127.14, 127.13, 127.09, 127.00, 126.61, 126.26, 126.12, 126.05, 125.94, 125.87, 125.83, 125.67, 125.51, 120.94, 114.79, 97.89, 94.36, 72.82, 72.43, 69.59, 68.74, 68.47, 67.51, 67.07, 65.21, 59.00, 58.36, 52.41, 23.30, 15.68, -7.38, -7.41. HRESI-MS: $C_{64}H_{63}NO_{17}Si$ (1145.3865). $[M+NH4]^+$ cald: 1163.4203, found: 1163.3753.

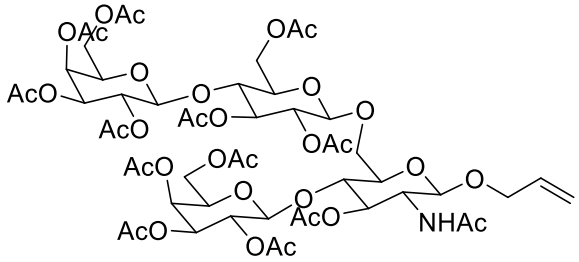


Compound 28: To a solution of compound **27** (0.2 g, 0.175 mmol) in DCM (5 mL) and acetonitrile (20 mL) in 50 mL plastic centrifuge tube was added aqueous HF (48%, 2 mL). The mixture was stirred at room temperature for 2 h, poured onto solid $NaHCO_3$ (10 g) in a conical flask. After 30 min, DCM (100 mL) was added. The organic phase was concentrated under reduced pressure. The crude product was subject to column chromatography (silica gel) with a mixture of EtOAc and hexane (1/1) as eluent, affording 0.153 g (85% yield) of clear oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.04-7.16 (m, 29 H), 6.16 (dd, J = 10.5, 8.5 Hz, 1 H), 5.78 (d, J = 3.5 Hz, 1 H), 5.74-5.66 (m, 1 H), 5.70 (t, J = 10.5 Hz, 1 H), 5.50 (dd, J = 10.0, 3.0 Hz, 1 H), 5.48 (d, J = 8.5 Hz, 1 H), 5.12 (ddd, J = 17.0, 1.5, 1.0 Hz, 1 H), 5.05 (ddd, J = 17.0, 1.5, 1.0 Hz, 1 H), 5.04 (d, J = 8.0 Hz, 1 H), 4.47 (dd, J = 10.5, 8.0 Hz, 1 H), 4.27 (t, J = 10.0 Hz, 1 H), 4.24 (tdd, J = 12.0, 5.0, 1.0 Hz, 1 H), 4.04 (dd, J = 10.5, 1.5 Hz, 1 H), 4.02 (tdd, J = 12.0, 5.0, 1.0 Hz, 1 H), 3.85-3.79 (m, 2 H), 3.676 (dd, J = 11.0, 7.0 Hz, 1 H), 3.61 (td, J = 10.0, 2.0 Hz, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 165.58, 165.47, 165.34, 165.24, 164.77, 134.09, 133.43, 133.34, 133.25, 133.19, 133.12, 129.96, 129.75, 129.72, 129.70, 129.64, 129.59, 129.43, 129.10, 128.94, 128.72, 128.55, 128.53, 128.49, 128.25, 128.23, 123.54, 117.78, 100.93, 97.39, 75.70, 74.81, 71.88, 71.08, 70.40, 70.12, 67.65, 60.91, 60.43, 54.89. HRESI-MS: $C_{58}H_{49}NO_{17}$ (1031.3000). $[M+NH4]^+$ cald: 1049.3338, found: 1049.3327.



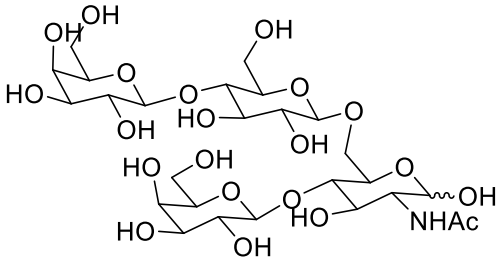
Compound 30: Donor **29** (71 mg, 0.091 mmol, freshly prepared by treatment of 2,3,6,2',3',4',6'-tetra-*O*-acetyl-D-lactose with trichloroacetonitrile in the presence of K_2CO_3), acceptor **28** (77.3 mg, 0.075 mmol), molecular sieves (4 Å, 400 mg) were mixed in DCM (6 mL). The mixture was stirred at room temperature for 1 h, followed by addition of TMSOTf (20 µL). The reaction was allowed to stirr overnight before Et_3N (0.1 mL) was

added. After filtration through a celite pad, the solvent was evaporated. The residue was purified by column chromatography (silica gel) using a mixture of EtOAc and hexane (1/1 to 3/1), affording a clear glass (100.2 mg, 81% yield). ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.97-7.15 (m, 29 H), 6.13 (dd, *J* = 11.0, 9.0 Hz, 1 H), 5.75 (d, *J* = 4.5 Hz, 1 H), 5.74-5.68 (m, 1 H), 5.679 (dd, *J* = 10.5, 8.0 Hz, 1 H), 5.50 (dd, *J* = 10.5, 3.5 Hz, 1 H), 5.8 (d, *J* = 8.5 Hz, 1H), 5.37 (dd, *J* = 4.5, 1.0 Hz, 1 H), 5,147 (dd, *J* = 10.5, 7.5 Hz, 1 H), 5.121 (ddd, *J* = 15.5, 2.5, 1.5 Hz, 1 H), 5.06 (ddd, *J* = 11.0, 2.5, 1.5 Hz, 1 H), 4.98 (dd, *J* = 12.0, 1.5 Hz, 1 H), 4.97 (t, *J* = 9.0 Hz, 1 H), 4.895 (dd, *J* = 10.0, 7.0 Hz, 1 H), 4.88 (d, *J* = 8.0 Hz, 1 H), 4.53 (dd, *J* = 11.5, 2.0 Hz, 1 H), 4.46 (t, *J* = 6.0 Hz, 1 H), 4.44 (t, *J* = 6.0 Hz, 1 H), 4.25 (tdd, *J* = 13.0, 5.0, 1.5 Hz, 1 H), 4.16 (d, *J* = 7.5 Hz, 1 H), 4.12 (t, *J* = 6.5 Hz, 1 H), 4.05-4.00 (m, 2 H), 3.98 (t, *J* = 8.0 Hz, 1 H), 3.90-3.83 (m, 3 H), 3.77 (dd, *J* = 11.0, 5.5 Hz, 1 H), 3.72 (t, *J* = 9.0 Hz, 1 H), 3.50 (dd, *J* = 11.5, 7.5 Hz, 1 H), 3.19 (ddd, *J* = 14.5, 6.5, 2.0 Hz, 1 H), 2.24 (s, 3 H), 2.17 (s, 3 H), 2.12 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 1.98 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 165.39, 165.12, 164.94, 164.83, 164.54, 164.33, 164.05, 160.30, 160.21, 160.12, 159.31, 128.85, 128.30, 128.23, 128.07, 127.97, 124.77, 124.54, 124.51, 124.40, 124.34, 124.21, 123.77, 123.67, 123.34, 123.11, 123.06, 118.33, 112.53, 110.00, 96.086 (2C), 95.78, 91.79, 72.44, 71.15, 69.05, 67.69, 67.63, 66.47, 66.35, 65.96, 65.80, 65.48, 65.02, 64.81, 63.84, 62.36, 61.47, 56.88, 55.61, 55.38, 49.56, 15.63, 15.44, 15.43, 15.42, 15.41. HRESI-MS: $C_{84}H_{83}NO_{34}$ (1649.4796). $[M+NH4]^+$ cald: 1667.5134, found: 1667.5163.

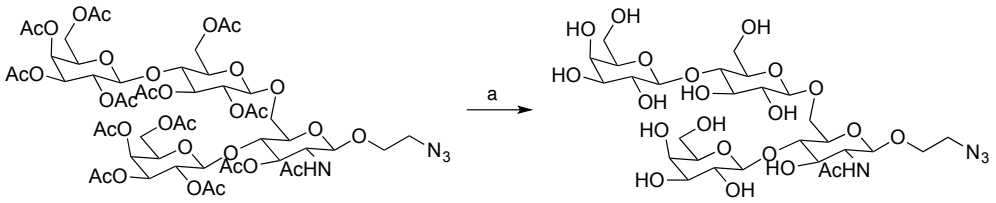


Compound 31: To a solution of compound **30** (100 mg, 0.061 mmol) in dry methanol (2 mL) and THF (8 mL) was added NaOMe (1 mL, 1 *M* in methanol). The mixture was stirred at room temperature for 2 h. After quencheding by acetic acid (0.1 mL), solvent was removed under reduced pressure. The residue was dissolved in dry methanol (9 mL). To this solution was added ethylene diamine (3.5 mL). The reaction was stirring at reflux overnight. The solvent was removed under reduced pressure. The residue was dissolved in dry pyridine (15 mL), followed by addition of acetic anhydride (8 mL) and DMAP (10 mg, 0.082 mmol). The mixture was stirred overnight, quenched by methanol (8 mL), diluted with DCM (180 mL), and washed with 1 N aqueous HCl (300 mL), a saturated aqueous $NaHCO_3$ solution, and brine. The organic phase was concentrated under vacuum. The product was purified by flash column chromatography (silica gel) using a mixture of methanol and DCM (0/1-1/9), affording a colorless oil (49 mg, 64% yield). ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.71 (d, *J* = 9.5 H, 1 H), 5.84 (m, 2 H), 5.35 (dd, *J* = 9.0, 3.0 Hz, 2 H), 5.27 (d, *J* = 17.5 Hz, 1 H), 5.18-5.15 (m, 2 H), 5.11-5.01 (m, 4 H), 4.95 (dd, *J* = 10.5, 3.5 Hz, 1 H), 4.88 (t, *J* = 9.0 Hz, 1 H), 4.59 (d, *J* = 7.5 Hz, 1 H), 4.49-4.44 (m, 3 H), 4.29 (dd, *J* = 13.5, 4.5 Hz, 1 H), 4.15-3.92 (m, 8 H), 3.88 (t, *J* = 7.0 Hz, 1 H), 3.83 (t, *J* = 9.5 Hz, 1 H), 3.78 (s, 1 H), 3.74-3.70 (m, 2 H), 3.62 (m, 1 H), 3.55 (m, 1 H), 2.19 (s, 3 H), 2.14 (s, 6 H), 2.11 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 6 H), 2.03 (s, 6 H), 2.02 (s, 3 H), 1.96 (s, 6 H), 1.95 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): 170.46, 170.35, 170.31, 170.13,

170.06, 169.99, 169.68, 169.61, 169.48, 169.08, 133.53, 117.33, 109.24, 101.10, 100.61, 100.52, 99.59, 75.99, 75.08, 74.40, 72.83, 72.60, 71.79, 71.67, 70.95, 70.58, 70.53, 69.37, 69.13, 69.06, 68.13, 66.78, 66.57, 62.01, 60.79, 60.71, 52.31, 38.87, 23.19, 21.07, 20.83, 20.79, 20.76, 20.73, 20.64, 20.62, 20.53, 20.50. HRESI-MS: $C_{53}H_{73}NO_{33}$ (1251.4065). $[M+NH4]^+$ cald: 1269.4409, found: 1269.4182.

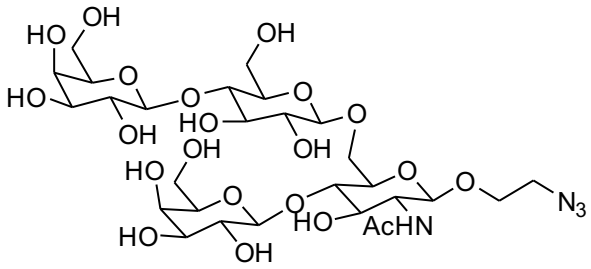


TS-14 Antigen: To a solution of compound **31** (49 mg, 0.039 mmol) in toluene (12 mL) and ethanol (6 mL) was added Wilkinson's catalyst (13 mg, 0.014 mmol). The reaction was stirred at reflux for 5 h. After removal of solvent under vacuum, the crude mixture was dissolved in THF (8 mL) and water (0.5 mL), followed by addition of NBS (0.1g). The mixture was stirred for 30 min, diluted with DCM (40 mL), then washed with a saturated aqueous Na_2SO_3 solution (100 mL). The organic layer was concentrated, and the product was purified by silica gel column chromatography using 40% EtOAc in hexane as eluent, affording 34 mg of crude product. This product was dissolved in THF (3 mL) and MeOH (3 mL), followed by addition of NaOMe (1 *M*, 0.1 mL). After stirring, the reaction mixture was quenched with AcOH (0.05 mL). The solvent was then evaporated, and the residue was subjected to silica gel column chromatography using a mixture of EtOAc, MeOH and water (60/25/20) as eluent, affording 11 mg (40% yield as a mixture of anomers) of **TS-14 Antigen** as a white solid. ^{1}H NMR (500 MHz; CD_3OD): δ 5.05 (d, J = 2.5 Hz, 1 H), 4.58 (d, J = 8.0 Hz, 1 H), 4.42-4.37 (m, 3 H), 4.30 (d, J = 8.0 Hz, 2 H), 4.05 (d, J = 11.5 Hz, 1 H), 3.95 (m, 1 H), 3.85-3.34 (m, 17 H), 3.26-3.20 (m, 2 H), 1.88 (s, 3 H). ^{13}C NMR (125 MHz, CD_3OD, ppm): 174.36, 111.00, 102.85, 102.69, 102.24, 94.93, 90.53, 78.27, 78.01, 75.27, 75.18, 74.62, 74.58, 74.18, 73.47, 72.56, 72.42, 72.39, 71.96, 70.86, 69.15, 68.98, 68.50, 68.46, 67.45, 62.38, 60.99, 60.93, 59.95, 56.05, 53.60, 22.08, 21.79. HRESI-MS: $C_{26}H_{45}NO_{21}$ (707.2484). $[M+NH_4]^+$ cald: 725.2864, found: 725.2820.

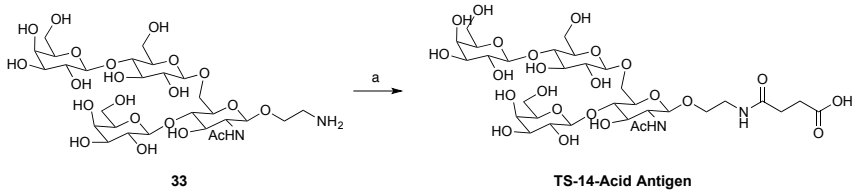


Reagents: a: THF, MeOH, H_2O, NaOMe, 77% yield.

Scheme 3. Synthesis of TS-14-N_3

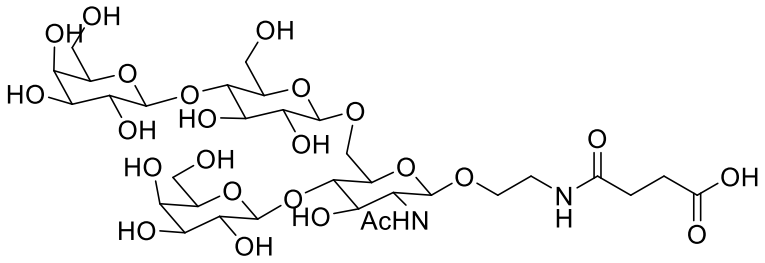


TS-14-N_3: To a solution of compound **32** (21 mg, 0.0164 mmol) in THF (3 mL), MeOH (3 mL) and water (0.1 mL) was added a solution of NaOMe in MeOH (1 *M*, 0.3 mL). The mixture was then stirred at room temperature overnight, followed by addition of AcOH (0.2 mL). After removal of solvent under reduced pressure, the crude product was subsequently purified by silica gel column chromatography using a mixture of solvents (EtOAc/MeOH/H2O: 60/25/20) as eluent, affording **TS-14-N_3** as a white solid (9.8 mg, 77% yield). ^{1}H NMR (500 MHz; CD_3OD): 4.53 (dd, *J* = 12.0, 2.5 Hz, 1 H), 4.49 (d, *J* = 8.5 Hz, 1 H), 4.46 (d, *J* = 8.0 Hz, 1 H), 4.36 (d, *J* = 7.5 Hz, 1 H), 4.24 (dd, *J* = 11.0, 1.5 Hz, 1 H), 4.008 (ddd, *J* = 11.0, 5.0, 3.0 Hz, 1 H), 3.95 (dd, *J* = 11.0, 4.0 Hz, 1 H), 3.90 (dd, *J* = 12.5, 2.5 Hz, 1 H), 3.84 (dd, *J* = 12.0, 4.0 Hz,1 H), 3.81-3.40 (m, 22 H), 3.32 (m, 1 H), 1.99 (s, 3 H). ^{13}C NMR (125 MHz, CD_3OD, ppm): 172.23, 103.66, 103.40, 103.11, 101.22, 79.09, 78.90, 75.66, 75.44, 75.03, 74.97, 74.02, 73.38, 73.29, 72.75, 71.21, 71.13, 68.95, 68.88, 68.09, 67.18, 61.08, 60.44, 55.13, 50.37, 21.66. HRESI-MS: $C_{28}H_{48}N_4O_{21}$ (776.2811). $[M+Na]^+$ cald: 799.2713, found: 799.2726.



Reagents: a: succinic anhydride, DMF, 76% yield.

Scheme 5. Synthesis of TS-14-Acid Antigen



TS-14-Acid Antigen: A mixture of compound **34** (15 mg, 0.02 mmol, prepared from compound **33** as described above), succinic anhydride (10 mg, 0.1 mmol), and DMF (5 mL) was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure, and the crude mixture was subjected to flash column chromatography (silica gel) using a mixture of EtOAc, MeOH and water (60/25/20) as the eluent, giving **TS-14-Acid Antigen** as a white powder (13 mg, 76% yield). ^{1}H NMR (500 MHz; D_2O): 4.39 (d, *J* = 7.5 Hz, 2 H), 4.38 (d, *J* = 7.5 Hz, 1 H), 4.30 (d, *J* = 7.5 Hz, 1 H), 4.14 (dd, *J* = 10.0, 1.5 Hz, 1 H), 3.84-3.36 (m, 23 H), 3.24-3.19 (m, 2 H), 2.30 (t, *J* = 4.5 Hz, 4 H), 1.88 (s, 3 H). ^{13}C NMR (125 MHz, D_2O, ppm): 175.82, 174.50, 102.84, 102.61, 102.31, 101.12, 78.27, 77.49, 75.24, 75.15, 74.60, 74.16, 73.33, 72.54, 72.41, 72.35, 72.19, 71.95, 70.85, 70.82, 68.45, 68.18, 62.36, 60.98, 60.91, 59.94, 54.99, 39.17, 32.91, 32.25, 22.08. HRESI-MS: $C_{32}H_{54}N_{52}O_{24}$ (850.3067). $[M+Na]^+$ cald: 873.3001, found: 873.2940.

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