Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Precise Immunological Evaluation Rationalized the Design of Self-Adjuvanting Vaccine Composed of Glycan Antigen, TLR1/2 Ligand, and T-helper Cell Epitope

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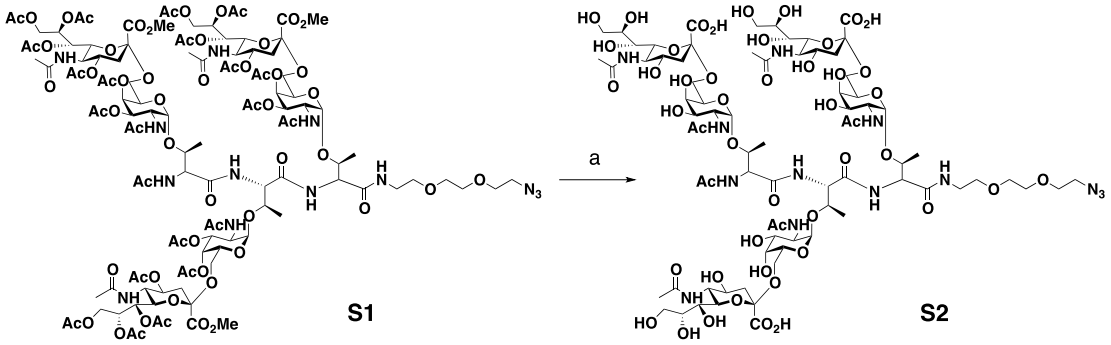
1. General information

^{1}H and ^{13}C NMR spectra were recorded in an indicated solvent with a 500 MHz spectrometer equipped with a cryoprobe. For ^{1}H NMR analysis, HDO (δ = 4.65 ppm) is used as an internal standard for the measurement in D_2O. CHD_2OD (δ = 3.30 ppm) is used as references for the measurements in CD_3OD. Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra were obtained on an ESI-LTQ-Orbitrap XL (FTMS) mass spectrometer. Unless otherwise noted, reactions in anhydrous solvent were carried out under Ar atmosphere. Distilled CH_2Cl_2 was distilled from calcium hydride. All other commercially available reagents and solvents were used as purchased.

Materials and reagents:

96 well Microwell™ MaxiSorp™ microtiter plates for the ELISA were purchased from Sigma Aldrich. Horseradish peroxidase (HRP)-linked goat anti-mouse IgG and IgM antibodies were purchased from Sigma Aldrich. HRP-linked goat anti-mouse IgG1, IgG2a, IgG2b, and IgG3 antibodies were purchased from Abcam. Alexa Fluro®488-goat anti-mouse IgG antibodies were purchased from Molecular Probes.

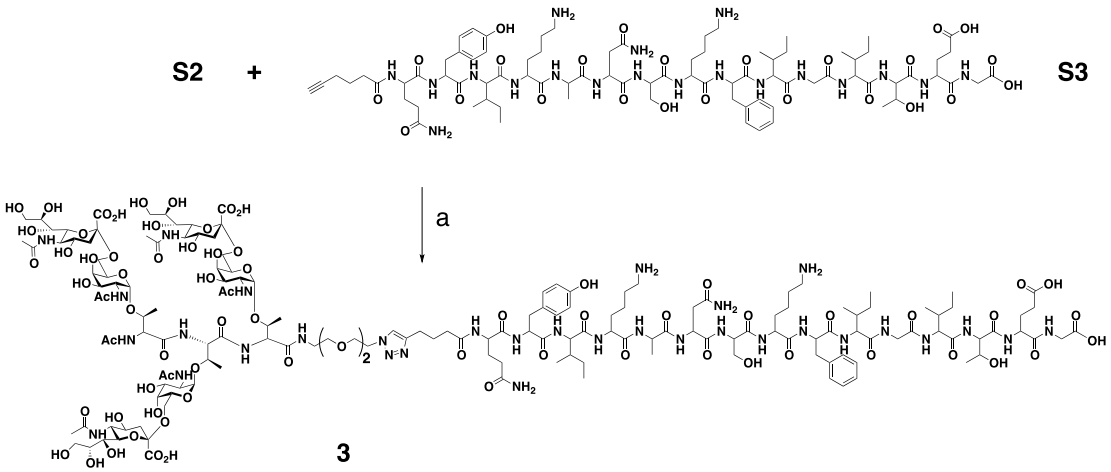
2. Synthesis procedures and characterization data for compounds 2 and 3.



Scheme S1. Synthesis of compound **S2**. (a) LiOH, MeOH, H_2O, rt, 14 h, 87%.

Compound S2

To a solution of compound **S1**[1] (28.0 mg, 9.99 x 10^{-3} mmol) in MeOH/H_2O (v/v = 1/1, 2.0 mL), and then was treated with lithium hydroxide monohydrate (25.1 mg, 0.60 x 10^{-1} mmol). After the solution was stirred at room temperature for 14 h, the reaction was neutralized with 90 mM $HOAc_{(aq)}$ and concentrated *in vacuo*. The crude product was purified by RP-HPLC on a Nacalai Tesque HILIC column (4.6 x 150 mm) at a flow rate of 1 mL/min using a mobile phase of 25 mM aqueous sodium acetate (Solvent A) and acetonitrile (Solvent B) (90% to 10% B gradient over 30 min, UV detection at 215 nm; retention time: 29.0 min) to afford **S2** as a white solid following lyophilisation (17.0 mg, 85%). ^{1}H NMR (500MHz, D_2O): δ 4.84 (d, J = 4.0 Hz, 1H), 4.77 (d, J = 4.0 Hz, 1H), 4.57 (d, J = 3.0 Hz, 1H), 4.42 (d, J = 9.0 Hz, 1H), 4.31-4.27 (m, 1H), 4.21-4.14 (m, 2H), 4.03-3.95 (m, 6H), 3.89-3.70 (m, 17H), 3.64-3.39 (m, 25H), 3.36-3.32 (m, 1H), 3.15-3.10 (m, 1H), 2.63-2.58 (m, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.92 (s, 9H), 1.90 (s, 3H), 1.62-1.55 (m, 3H), 1.26-1.22 (m, 4H), 1.16 (d, J = 6.0 Hz, 3H). HRMS (ESI-LTQ-Orbitrap XL, positive) calcd for $C_{77}H_{129}N_{13}O_{48}$ $[M+2H]^{2+}$: 1001.9021. Found: 1001.9069.



Scheme S2. Synthesis of compound **3**. (a) $CuSO_4$, Sodium ascorbate, 80% DMSO in H_2O, rt, 3 h, 53%.

Compound 3

$CuSO_4$ (96 μg, 6.0 x 10^{-4} mmol) and sodium L-ascorbate (357 μg, 1.8 x 10^{-3} mmol) were mixed in oxygen-free water (57 μL). And then, the resultant solution was added to a solution of compound **S2** (1.0 mg, 5.0 x 10^{-4} mmol) and **S3**[1] (1.05 mg, 6.0 x 10^{-4} mmol) in oxygen-free DMSO (255 μL) under Ar atmosphere at room temperature. After the mixture was stirred for 3.0 h at this temperature, DOTA (1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid, 2.42 mg, 6.0 x 10^{-3} mmol) was added, and the resulting solution was stirred for another 20 min. The mixture was lyophilized prior to purification. The crude product was purified by RP-HPLC on a Nacalai Tesque 5C_{18}-AR300 column (4.6 x 250 mm) at a flow rate of 1 mL/min using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) (1 to 100% B gradient over 180 min; UV detection at 215 nm; retention time: 46.1 min) to afford **3** as a white solid following lyophilisation (1.0 mg, 53%). The compound **3** was analyzed by analytical RP-HPLC (see Figure S1). ^{1}H NMR (500MHz, D_2O): 7.79 (s, 1H), 7.30-7.23 (m, 3H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.73 (d, *J* = 8.0 Hz, 2H), 4.89 (d, *J* = 3.5 Hz, 1H), 4.83-4.81 (m, 1H), 4.77-4.73 (m, 2H), 4.59-4.51 (m, 3H), 4.45 (s, 1H), 4.38-4.31 (m, 4H), 4.26-4.01 (m, 14H), 3.97-3.74 (m, 24H), 3.67-3.51 (m, 17H), 3.45-3.38 (m, 3H), 3.13-3.05 (m, 2H), 3.00-2.70 (m, 7H), 2.67-2.64 (m, 8H), 2.42-2.39 (m, 2H), 2.29-2.12 (m, 4H), 2.10-1.92 (m, 18H), 1.91-1.53 (m, 15H), 1.44-1.00 (m, 25H), 0.87-0.70 (m, 17H). HRMS (ESI-LTQ-Orbitrap XL, positive, see Figure S2) calcd for $C_{159}H_{257}N_{32}O_{72}$ $[M+3H]^{3+}$: 1255.9150. Found: 1255.9153.

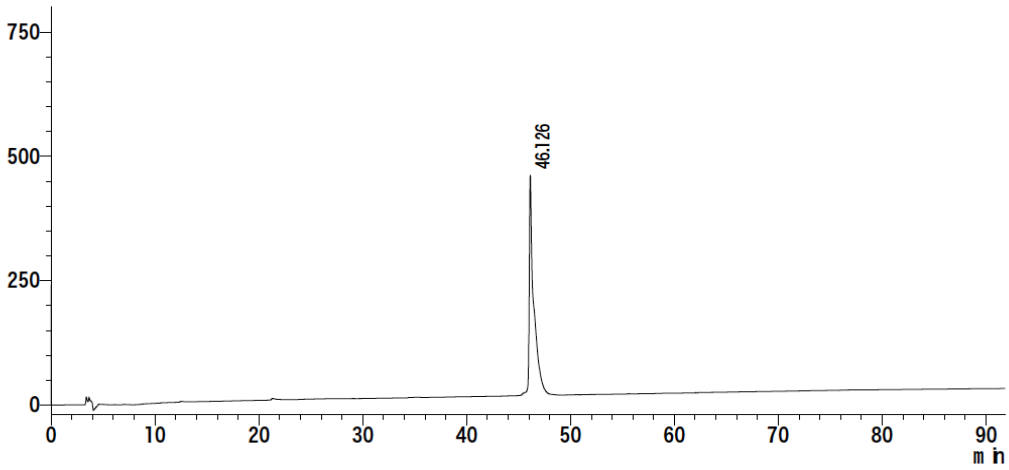


Figure S1. HPLC data of compound **3** by analytical column (Nacalai Tesque 5C$_{18}$-AR300, 4.6 x 250 mm; MeCN in H_2O containing 0.1% TFA (1-100% gradient over 180 min, 1 mL/min); UV detection at 215 nm).

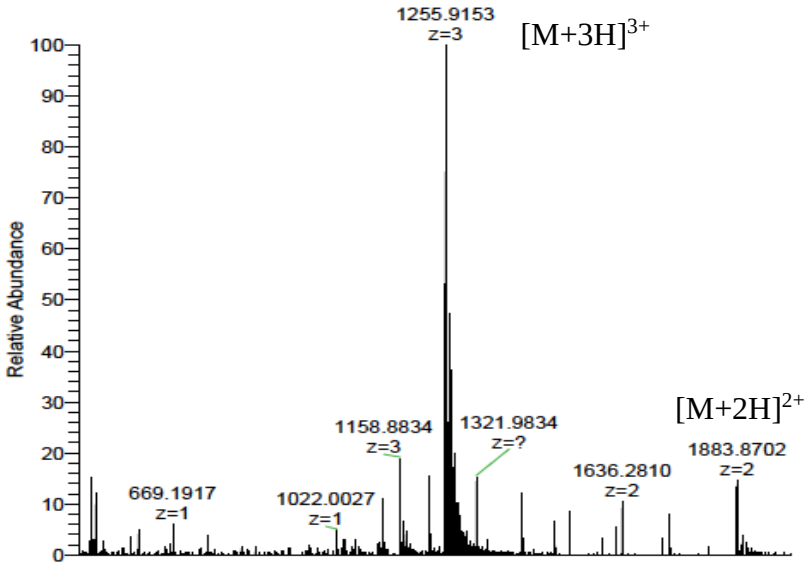
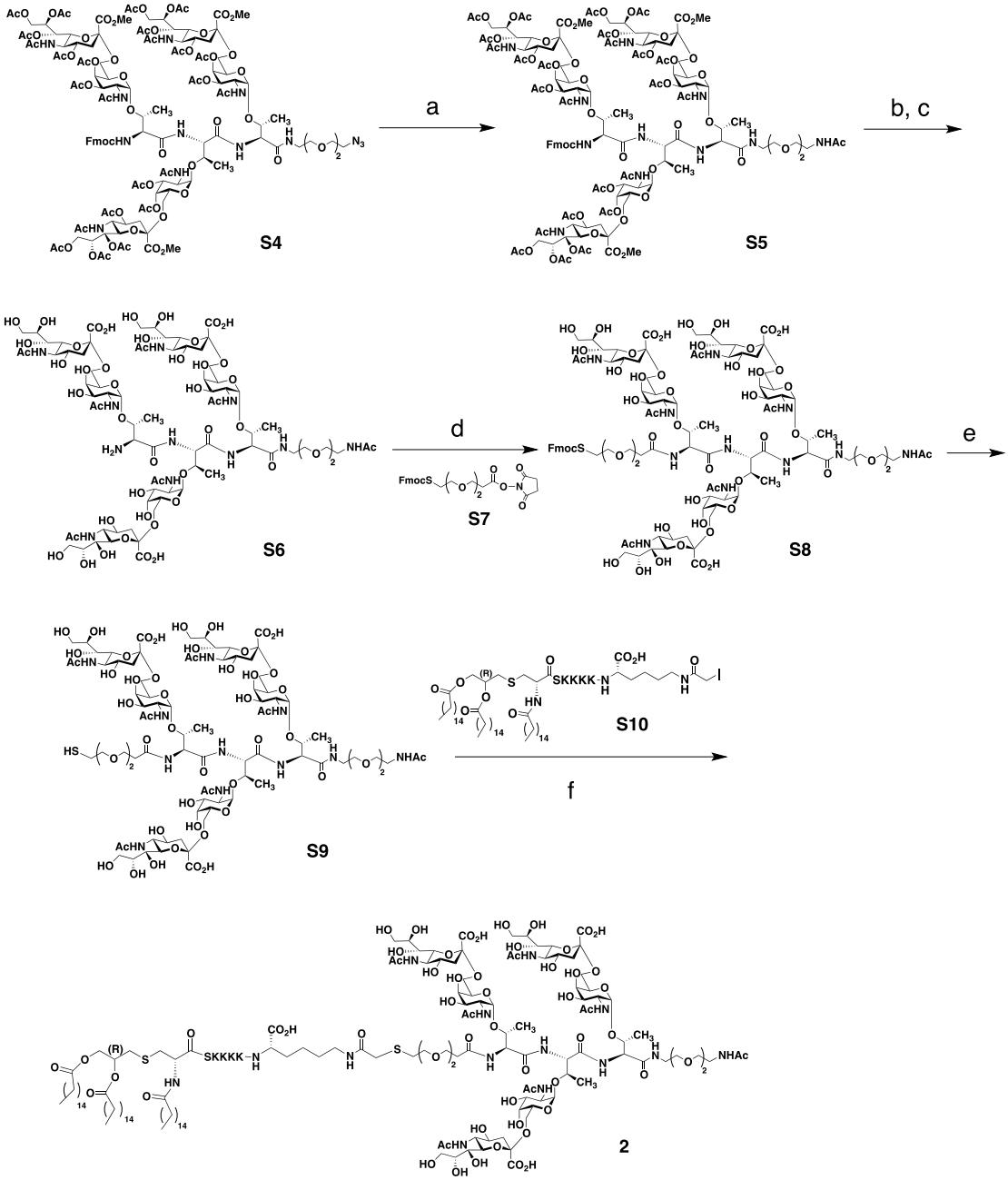


Figure S2. ESI-LTQ-Orbitrap MS data of compound **3**.



Scheme S3. Synthesis of compound **2**. (a) Zn, Ac_2O, HOAc, THF, 40 ºC, 6 h, 90% (b) 20% piperidine/DMF, rt, 20 min; (c) LiOH, MeOH, H_2O, rt, 14 h, 91% (2 steps); (d) **S7**, NMM, CH_3CN, H_2O, rt, 13 h, 67%; (e) (I) 50 mM LiOH, H_2O, rt, 30 min, and then acidification by 90 mM HOAc/H_2O; (II) 20 mM TCEP, H_2O, rt, 30 min, 81% (2 steps) (f) **S10**, Et_3N, DMSO, 40 ºC, 13 h, 47%.

Compound S5

To a solution of the compound **S4**[1] (28.0 mg, 9.99 x 10^{-3} mmol) in AcOH/THF (v/v = 1:1, total 1.0 mL) was added Zu powder (26.0 mg, 0.4 mmol) and acetic anhydrate (0.3 ml) at room temperature. After the mixture was stirred for 6 h at room temperature, the

Zn powder was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was extracted with AcOEt and the organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and then concentrated *in vacuo* to give crude product. The residue was purified with silica-gel column chromatography (MeOH/$CHCl_3$ = 1/10) to achieve compound **S5** as a white syrup (27.0 mg, 90 %). R_f 0.20 (MeOH/$CHCl_3$ = 1/10). ^{1}H NMR (500MHz, CD_3OD): δ 7.82 (d, J = 7.0 Hz, 2H), 7.70 (t, J = 7.0 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 5.38-5.29 (m, 10H), 5.21-5.14 (m, 2H), 5.10-5.03 (m, 2H), 4.97-4.94 (m 2H), 4.83-4.60 (m, 4H), 4.69 (d, J = 1.5 Hz, 1H), 4.59-4.55 (m, 1H), 4.50-4.39 (m, 5H), 4.32-4.24 (m, 9H), 4.18-4.03 (m, 9H), 3.98-3.90 (m, 3H), 3.88-3.77 (m, 12H), 3.62-3.46 (m, 9H), 3.40-3.38 (m, 2H), 3.30-3.27 (m, 2H), 3.12-3.08 (m, 1H), 2.61-2.51 (m, 3H), 2.16-2.14 (m, 9H), 2.10-2.05 (m, 21H), 1.99-1.96 (m, 27H), 1.93-1.90 (m, 9H), 1.85-1.72 (m, 12H), 1.37-1.27 (m, 9H). ^{13}C NMR (125 MHz, CD_3OD): δ 173.6, 173.4, 173.2(x2), 172.7(x2), 172.3(x2), 172.2(x2), 172.1, 172.0(x3), 171.7(x3), 171.5, 171.4(x3), 169.1(x2), 169.0, 145.4, 145.1, 142.7(x2), 128.8(x2), 128.2(x2), 126.2, 126.1, 121.0, 120.9, 101.0, 100.6, 99.9(x2), 79.6, 78.4, 78.3, 73.2(x2), 71.3, 71.2, 70.6(x2), 70.4(x2), 70.3, 69.3(x2), 69.1, 69.0, 68.4(x3), 67.7, 64.7, 64.6(x2), 63.3(x2), 60.2, 58.4, 58.1, 53.4(x2), 50.0, 40.5, 38.9, 38.8, 38.7, 23.5, 23.3(x2), 22.7, 22.6, 21.2, 21.0, 20.9(x2), 20.8(x2), 20.7(x2), 19.7, 19.6, 19.5. HRMS (ESI-LTQ-Orbitrap XL, positive) calcd for $C_{131}H_{181}N_{11}O_{68}Na_3$ $[M+3Na]^{3+}$: 1022.0251. Found: 1022.0253.

Compound S8

The compound **S5** (30.0 mg, 1.0 x 10^{-2} mmol) was treated with 20% piperidine in DMF (0.5 mL) at room temperature. The solution was then stirred for 20 min at room temperature. Evaporation of DMF in *vacuo* was followed by purification by flash chromatography on silica gel to give the amine of triSTn. To a solution of the amine of triSTn in MeOH/H_2O (v/v = 1/1, 8.0 mL) was treated with lithium hydroxide monohydrate (16.8 mg, 0.4 mmol) at room temperature. After the solution was stirred at room temperature for 14 h, the reaction was neutralized with 90 mM $HOAc_{(aq)}$ and concentrated *in vacuo*. The crude product was purified by RP-HPLC on a Nacalai Tesque 5C_{18}-AR300 column (4.6 x 250 mm) at a flow rate of 1 mL/min using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) (1% to 100% B gradient over 150 min; UV detection at 215 nm; retention time: 11.2 min) to afford **S6** as a white solid following lyophilisation (18.0 mg, 91% for two steps). The compound **S6** was analyzed by analytical RP-HPLC (see Figure S3). LRMS (ESI-LTQ-

Orbitrap XL, positive) calcd for $C_{77}H_{131}N_{11}O_{48}$ $[M+2H]^{2+}$: 988.9068. Found: 988.9392. To a solution of compound **S6** (18.0 mg, 9.11 x10^{-3} mmol) in acetonitrile (3.0 mL) and distilled water (3.0 mL) was added *N*-methylmorpholine (4 μL, 3.82 x10^{-2} mmol) and the linker **S7**[1] (19.6 mg, 3.82 x 10^{-2} mmol). After the solution was stirred at room temperature for 12 h, the mixture was concentrated *in vacuo*. The crude product was purified by RP-HPLC on a Nacalai Tesque 5C_{18}-AR300 column (4.6 x 250 mm) at a flow rate of 1 mL/min using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) (1% to 100% B gradient over 150 min; UV detection at 215 nm; retention time: 46.1 min) to afford **S8** as a white solid following lyophilisation (14.5 mg, 67%). ^{1}H NMR (500MHz, D_2O): δ 7.82-7.80 (m, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.42-7.38 (m, 2H), 7.35-7.31 (m, 2H), 4.86-4.78 (m, 3H), 4.72 (d, J = 3.5 Hz, 1H), 4.41-4.39 (m, 1H), 4.32-4.20 (m, 3H), 4.13 (d, J = 6.5 Hz, 1H), 4.01-3.92 (m, 5H), 3.88-3.65 (m, 21H), 3.60-3.32 (m, 28H), 3.28 (t, J = 5.0 Hz, 2H), 3.23-3.17 (m, 2H), 3.12-3.06 (m, 1H), 2.68-2.49 (m, 7H), 1.97 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.92 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H), 1.89 (s, 3H), 1.60-1.50 (m, 3H), 1.23 (d, J = 6.0 Hz, 3H), 1.17 (d, J = 6.5 Hz, 3H), 1.13 (d, J = 6.5 Hz, 3H). HRMS (ESI-LTQ-Orbitrap XL, positive) calcd for $C_{99}H_{151}N_{11}O_{53}SNa_2$ $[M+2Na]^{2+}$: 1210.4499. Found: 1210.4491.

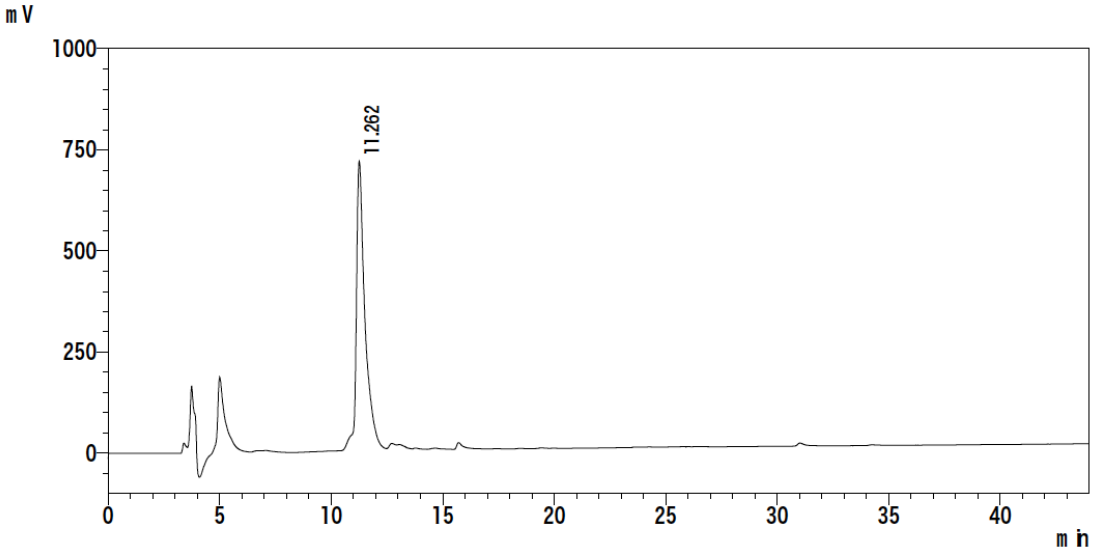


Figure S3. HPLC data of compound **S6** by analytical column (Nacalai Tesque 5C_{18}-AR300, 4.6 x 250 mm; MeCN in H_2O containing 0.1% TFA (1-100% gradient over 150 min, 1 mL/min); UV detection at 215 nm).

Compound S9

To a solution of compound **S8** (6.2 mg, 2.60 x 10^{-3} mmol) in MeOH/H_2O (v/v = 1/1,

1.18 mL) was treated with lithium hydroxide monohydrate (2.47 mg, 5.9 x 10^{-2} mmol) at room temperature. After the solution was stirred at room temperature for 30 min, the reaction was neutralized with 90 mM $HOAc_{(aq)}$ and concentrated *in vacuo*. The residue was dissolved in aqueous 20 mM tris(2-carboxyethyl)phosphine hydrochloride (2.0 mL). After 40 min, the solution was subjected to purification by RP-HPLC on a Nacalai Tesque 5C_{18}-AR300 column (4.6 x 250 mm) at a flow rate of 1.0 mL/min using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) (1 to 1% B isocratic over 50 min; UV detection at 215 nm; retention time: 16.5 min) to afford **S9** as a white solid following lyophilisation (4.5 mg, 81%). The compound **S9** was analyzed by analytical RP-HPLC (see Figure S4). HRMS (ESI-LTQ-Orbitrap XL, positive) calcd for $C_{84}H_{143}N_{11}O_{51}S$ $[M+2H]^{2+}$: 1076.9322. Found: 1076.9331.

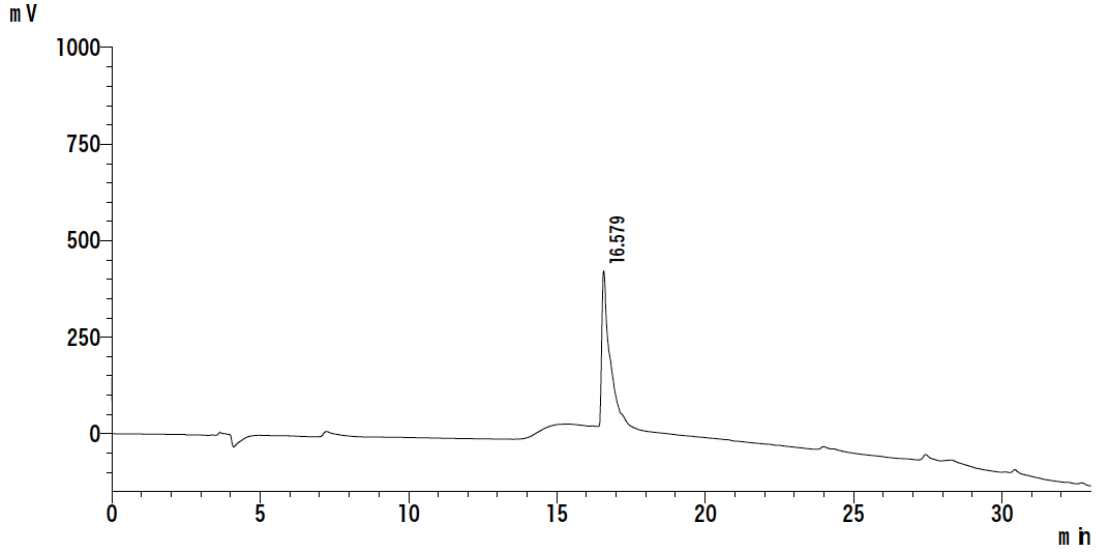


Figure S4. HPLC data of compound **S9** by analytical column (Nacalai Tesque 5C_{18}-AR300, 4.6 x 250 mm; MeCN in H_2O containing 0.1% TFA (1-1% isocratic over 50 min, 1 mL/min); UV detection at 215 nm).

Compound 2

To a solution of compound **S9** (3.50 mg, 1.62 x 10^{-3} mmol) and **S10**[1] (2.35 mg, 1.30 x 10^{-3} mmol) in DMSO (1.6 mL) was treated with triethylamine (266 nL, 1.62 x 10^{-3}

mmol) under Ar atmosphere. After the reaction was reacted at 40 °C for 15 h under Ar atmosphere, the mixture was concentrated to dryness *in vacuo*. The residue was purified by RP-HPLC on a Nacalai Tesque 5C$_4$-AR300 column (4.6 x 250 mm) at a flow rate of 1 mL/min using a mobile phase of 0.1% TFA in water/acetonitrile/isopropanol (v/v/v = 8/1/1, Solvent A) and 0.1% TFA in acetonitrile/isopropanol (v/v = 1/1, Solvent B) (50 to 100% B gradient over 120 min; UV detection at 215 nm; retention time: 51.6 min) to afford **2** as a white solid following lyophilisation (2.91 mg, 47%). The compound **2** was analyzed by analytical RP-HPLC (see Figure S5). HRMS (ESI-LTQ-Orbitrap XL, positive, see Figure S6) calcd for $C_{173}H_{312}N_{23}O_{66}S_2$ $[M+3H]^{3+}$: 1278.0419. Found: 1278.0486

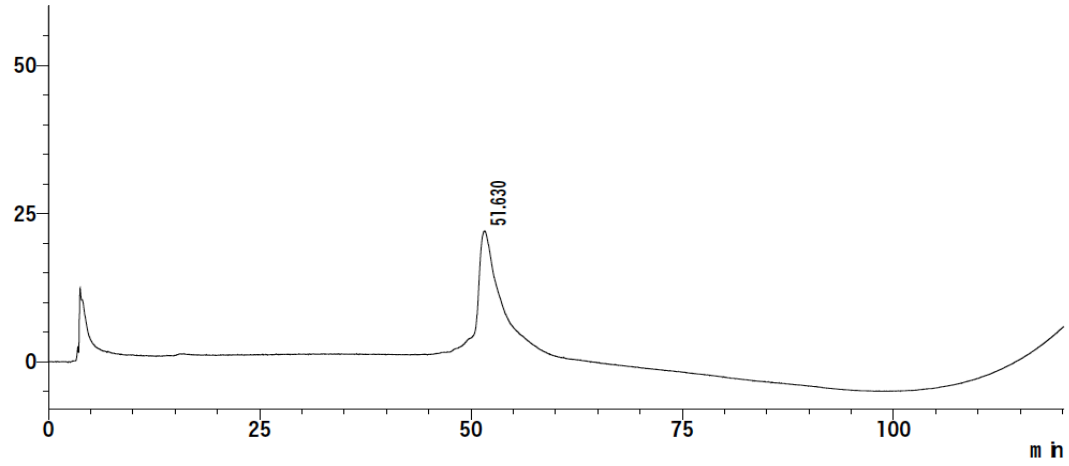


Figure S5. HPLC data of compound **2** by analytical column (Nacalai Tesque 5C$_4$-AR300, 4.6 x 250 mm; solvent A: 0.1% TFA in water/acetonitrile/isopropanol (v/v/v = 8/1/1) and solvent B: 0.1% TFA in acetonitrile/isopropanol; (50-100% gradient B over 120 min, 1 mL/min); UV detection at 215 nm).

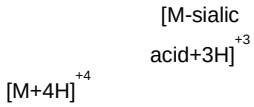


Figure S6. ESI-LTQ-Orbitrap MS data of compound **2**.

3. Immunization procedure and ELISA method

Mouse immunization

Male BALB/c mice were obtained from Tokai University, School of Medicine. Experiments using BALB/c mice were approved in compliance with the Guidelines for the Care and Use of Laboratory animals, and all animal studies were approved by the committees of the Tokai University School of Medicine. The numbers of approval are #185018 and #191075. Each group of five female wild-type BALB/c mice (8 weeks age) were inoculated with intraperitoneally (i.p.) injection of vaccine **1** (3.5 nmol), **2** (3.5 nmol), or **3** (3.5 nmol) diluted in 0.1 mL PBS on day 1. The immunization schedule included boosting each mouse with three times on days 14, 28, and 42, respectively, by injection of the same vaccine preparation. As for the V1', the first immunizations were performed with complete Freund's adjuvant (CFA), the others with incomplete Freund's adjuvant (IFA). Blood were collected from each mouse before immunization on day 0 (blank controls) and on day 8, 21, 35, and 49, and were clotted to obtain plasmas that were stored at -80 ^{o}C before use.

Protocol for ELISA

ELISA plates were coated with a solution of the triSTn-BSA, *N*-propionyl-triSTn-BSA, STn-BSA, linker-BSA, or BSA (10 μg /mL, 50 μL per well) in the coating buffer (50 mM carbonate, pH 9.5) at 4 ^{o}C for 16 h. Nonspecific sites were blocked with 1% BSA in coating buffer at 37 ^{o}C for 2 h, then washed three times with phosphate-buffered saline (PBS) at pH 7.4. Subsequently, an individual mouse plasma with serial half-log dilutions from 1:100 to 1:102400 in PBS containing 1% BSA were added to the coated plates (50 μL per well). The plates were incubated at 25 ^{o}C for 2 h and then washed three times with PBS containing 0.05 % Tween-20 (PBST) and incubated at room temperature for 1 h with a 1:1000 diluted solution of HRP-linked goat anti-mouse IgG or IgM antibody or a 1:3000 diluted solution of HRP-linked goat anti-mouse IgG1, IgG2a, IgG2b, or IgG3 antibody (50 μL per well), respectively. After the plates were washed five times with 0.05% PBST, 0.4 mg/mL *o*-phenylenediamine dihydrochloride (OPD) in 0.05 M phosphate-citrate buffer at pH 5.0 with 0.4 μL/mL 30% H_2O_2 was added to the plates (100 μL per well), and after 25 min at room temperature, 2.5 M aqueous sulfuric acid (50 μL per well) was added to stop the colorimetric reaction. Optical density (OD) was measured at 492 nm on an ELISA plate reader. For titer analysis, the OD values were plotted against the serum dilution numbers to obtain a

best-fit logarithm line. The equation of this line was used to calculate the dilution number at which an OD value of 0.1 was achieved, and this dilution number is defined as the antibody titer.

4. Detailed data of ELISA

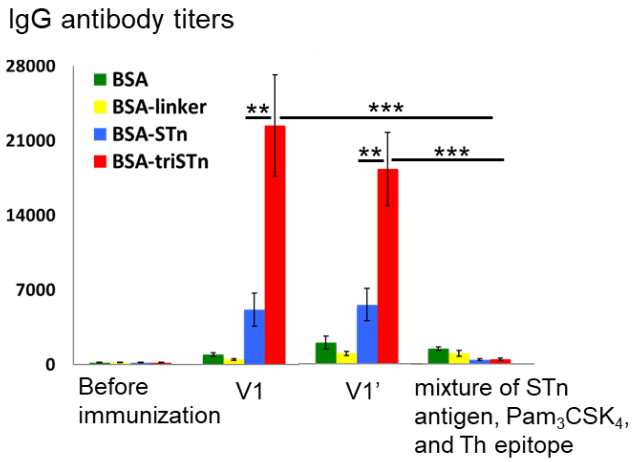


Fig. S7. IgG antibody titers induced by the immunization of V1, V1' and the mixture of STn antigen, Pam3CSK4, and Th epitope. [1] **: $p<0.01$, ***: $p<0.005$

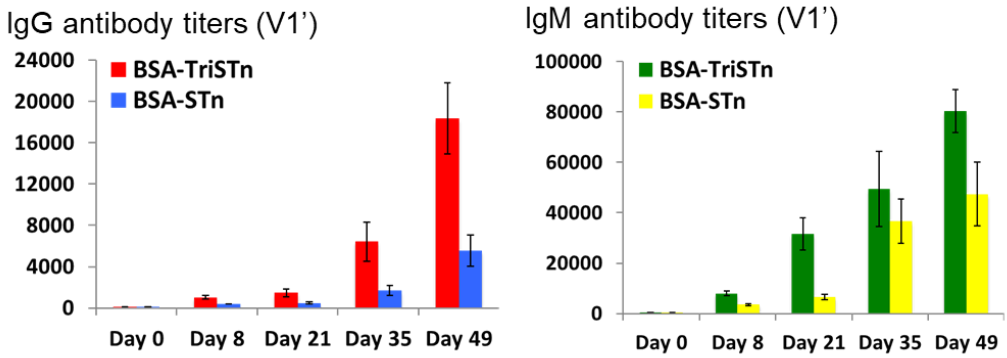


Fig. S8. IgG antibody titers induced by V1' on days 0, 7, 35, and 49. [1]

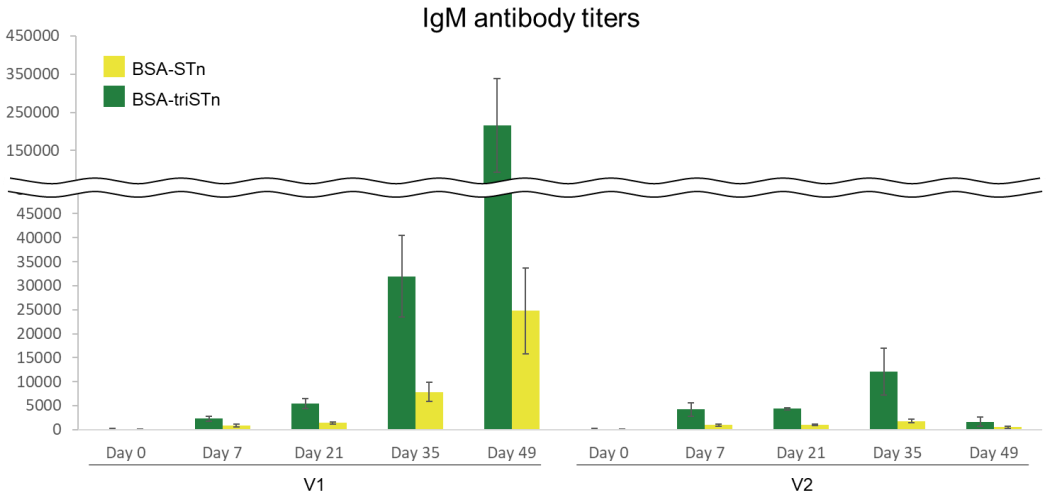


Fig. S9. IgM antibody titers induced by V1 and V2 on days 0, 7, 35, and 49.

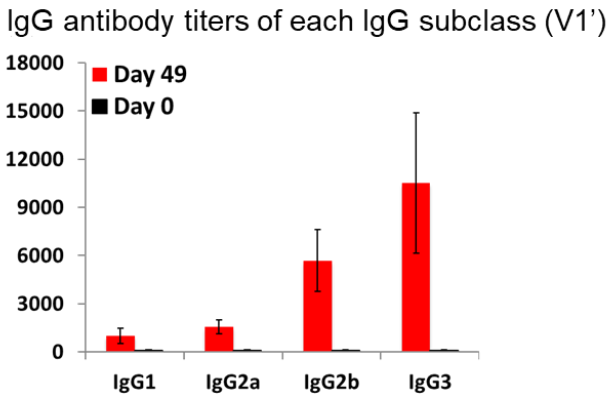


Fig. S10. IgG antibody titers of each IgG subclass on day 49 induced by V1'. [1]

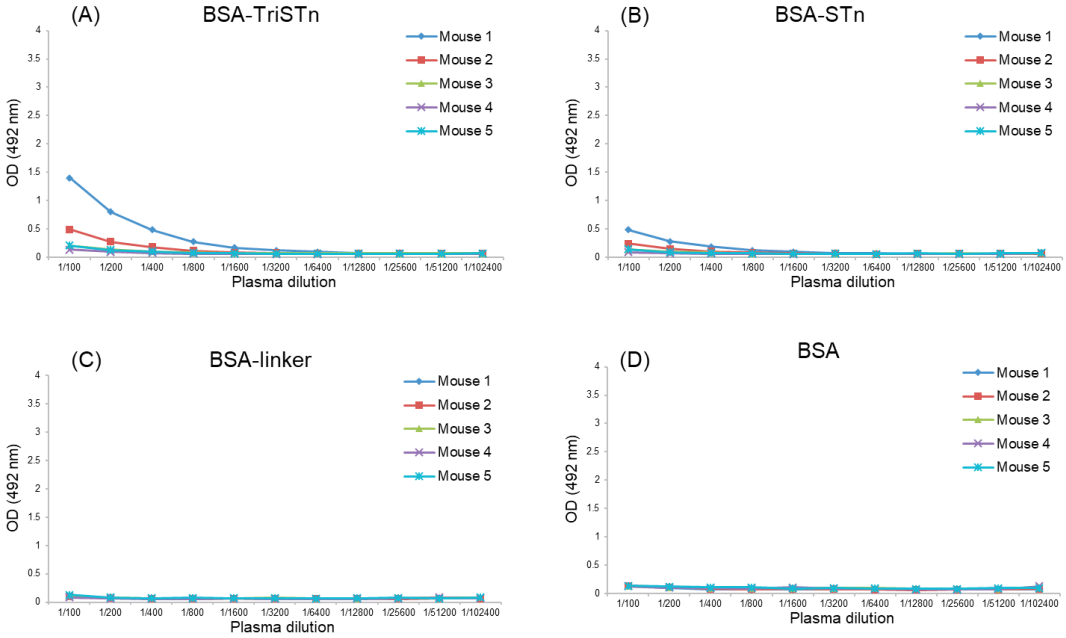


Fig. S11. ELISA of IgG antibody induced by V1 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

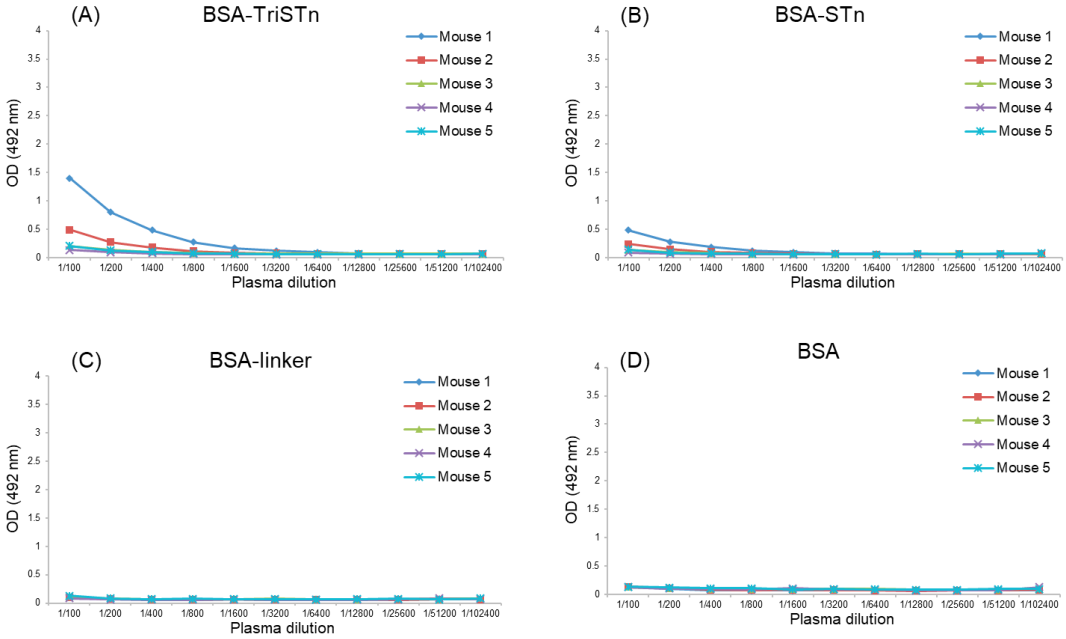


Fig. S12. ELISA of IgG antibody induced by V2 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

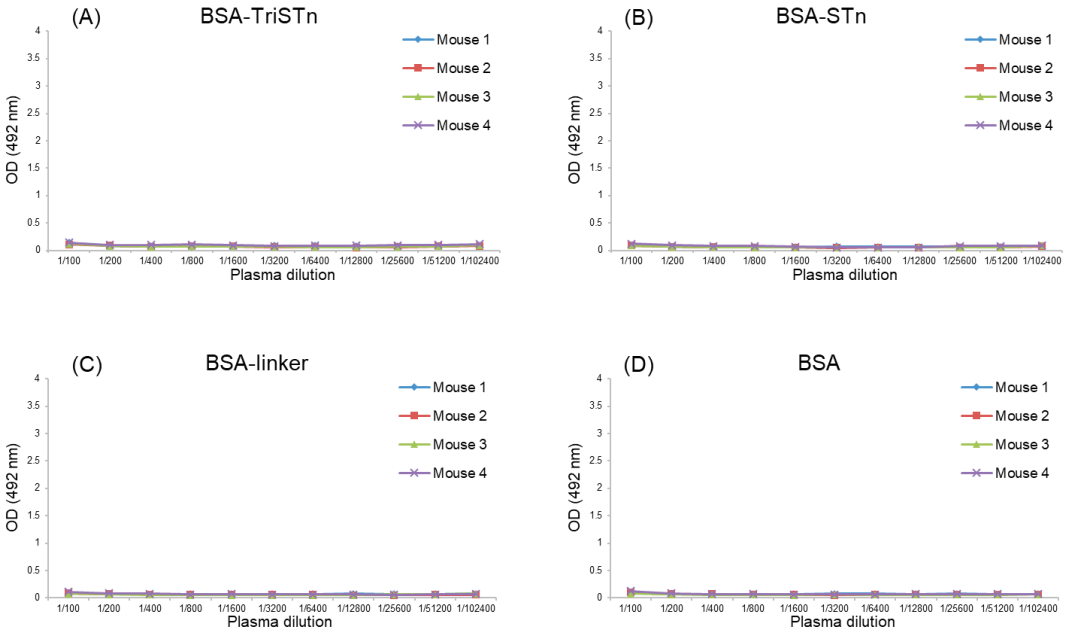


Fig. S13. ELISA of IgG antibody induced by V3 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

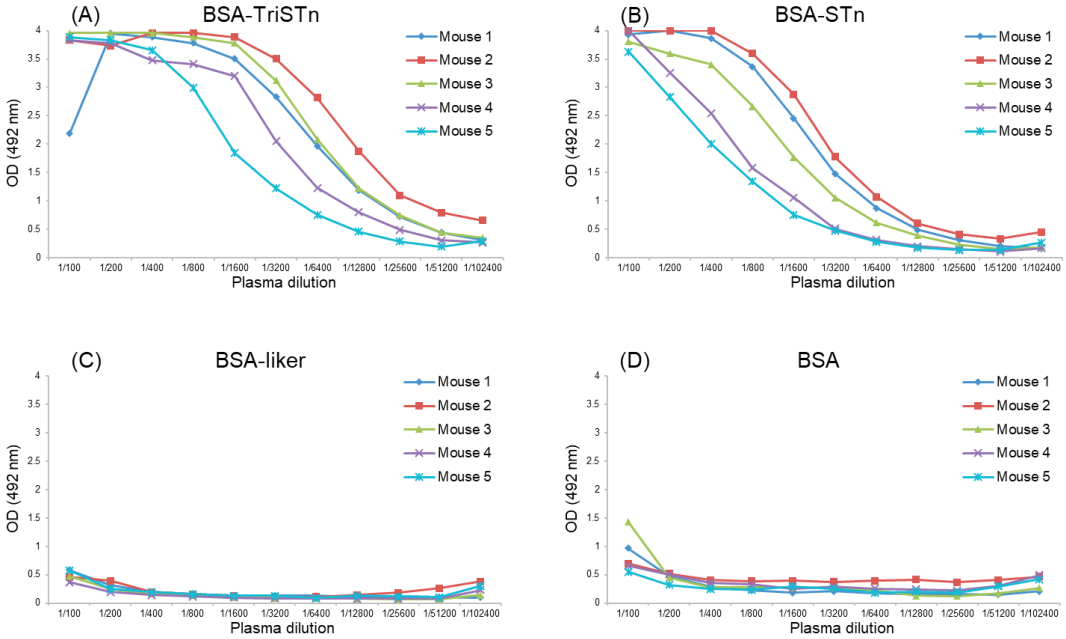


Fig. S14. ELISA of IgM antibody induced by V1 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

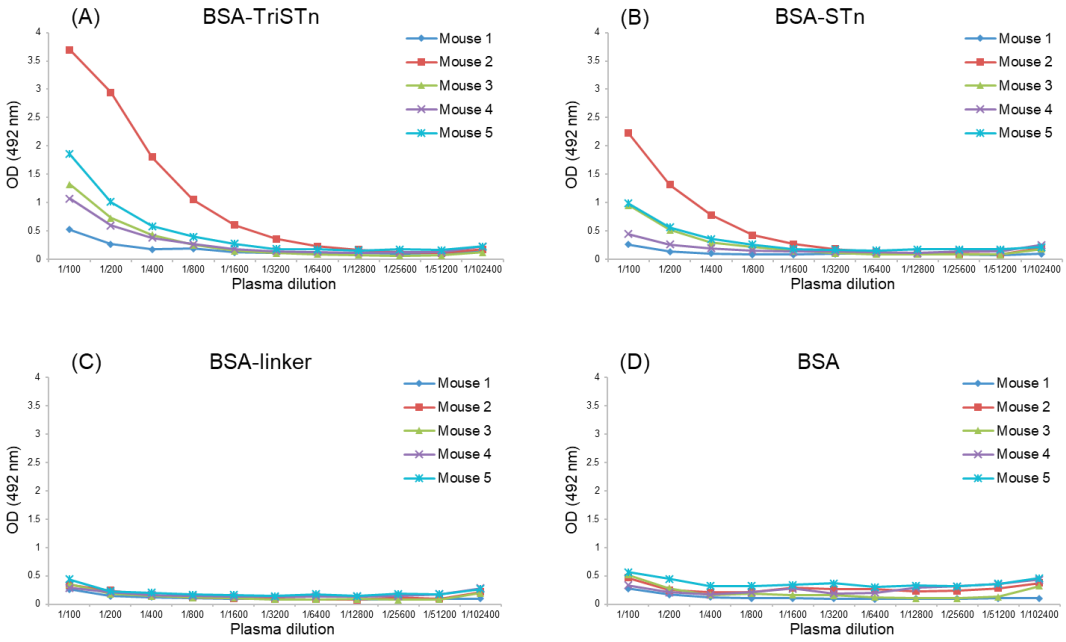


Fig. S15. ELISA of IgG antibody induced by V2 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

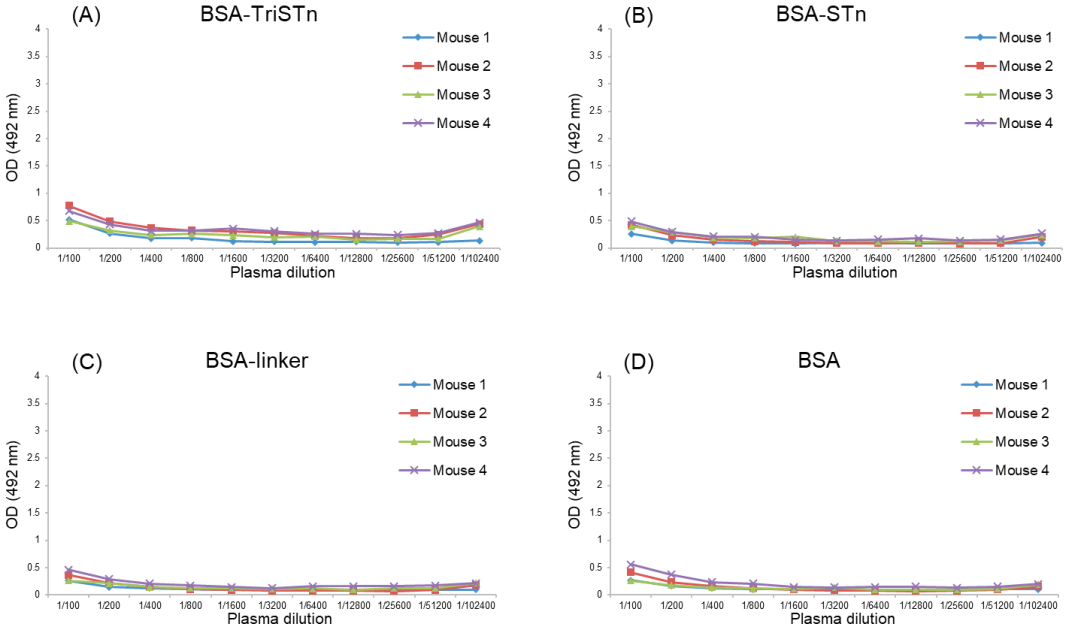


Fig. S16. ELISA of IgG antibody induced by V3 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

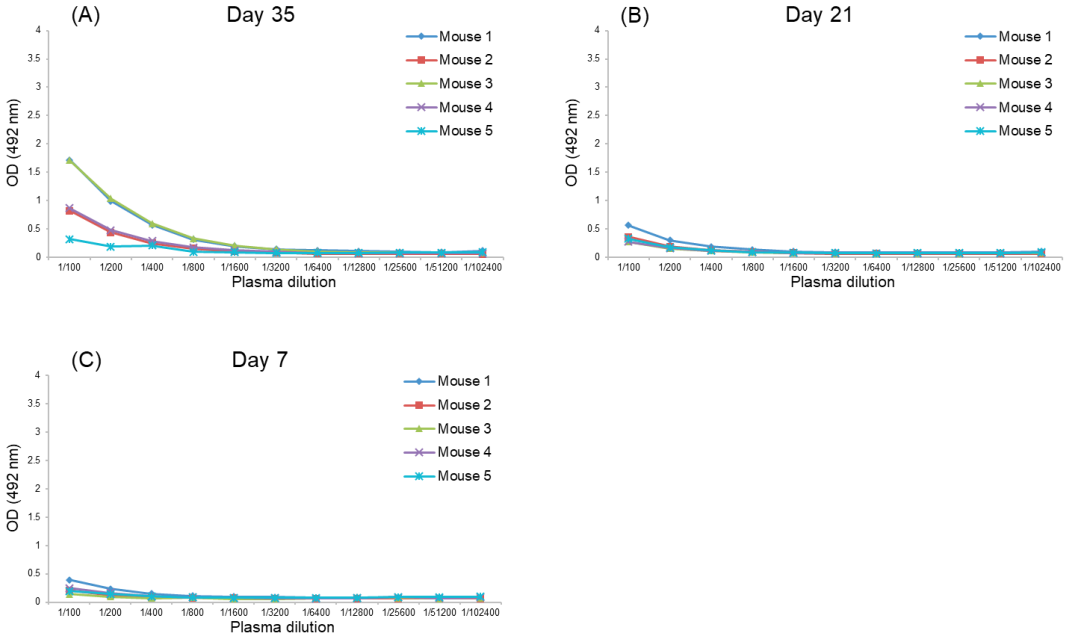


Fig. S17. ELISA of IgG antibody induced by V1 specific for BSA-STn on day A) 35, B) 21, or C) 7 of the immunization schedule.

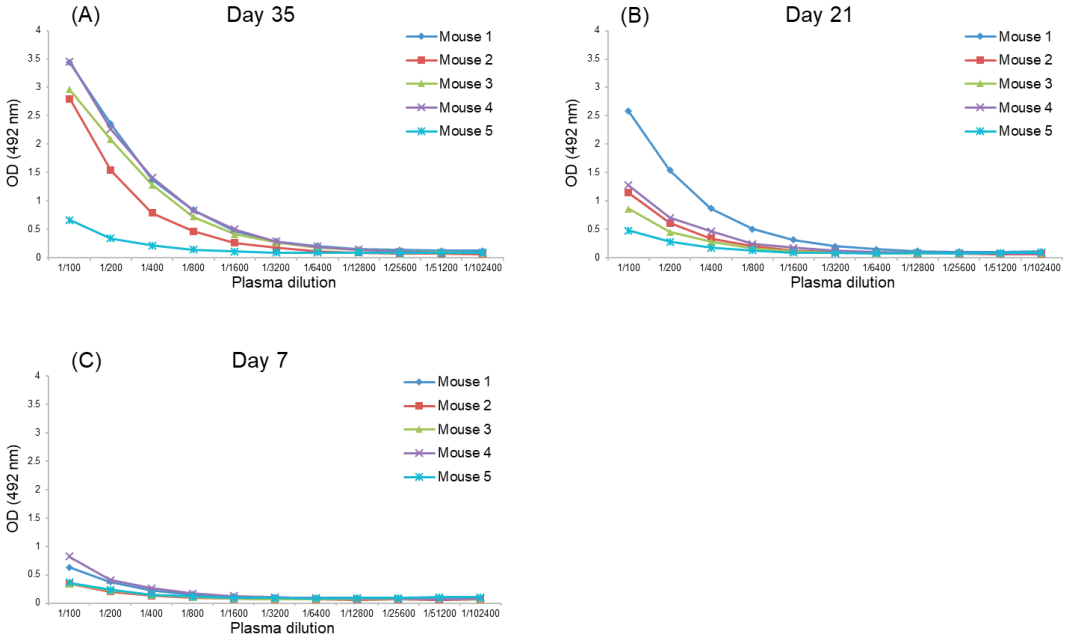


Fig. S18. ELISA of IgG antibody induced by V1 specific for BSA-triSTn on day A) 35, B) 21, or C) 7 of the immunization schedule.

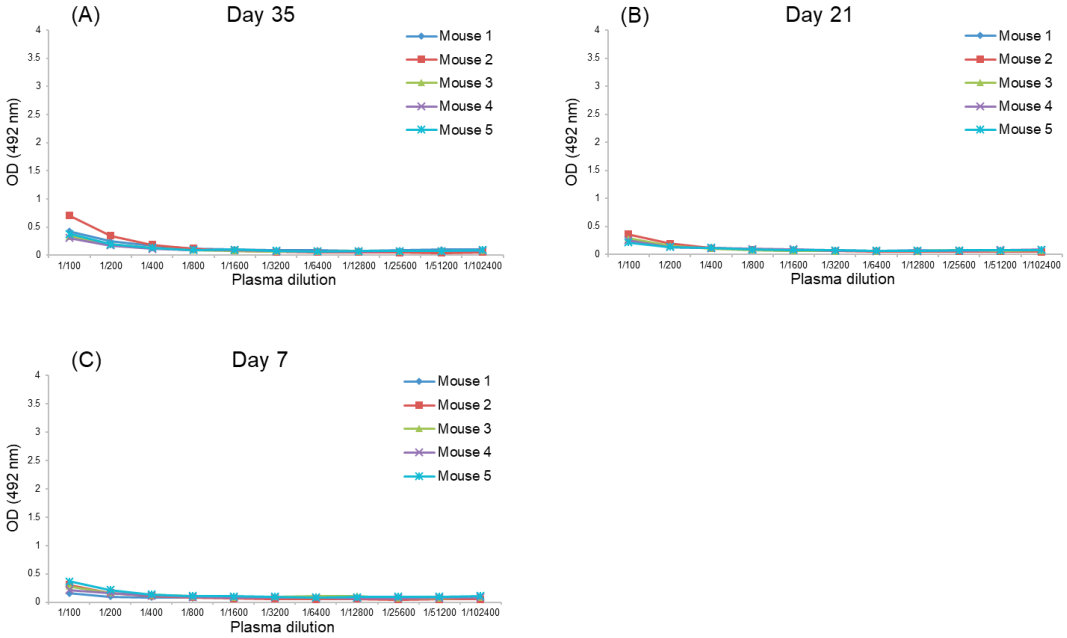


Fig. S19. ELISA of IgG antibody induced by V2 specific for BSA-STn on day A) 35, B) 21, or C) 7 of the immunization schedule.

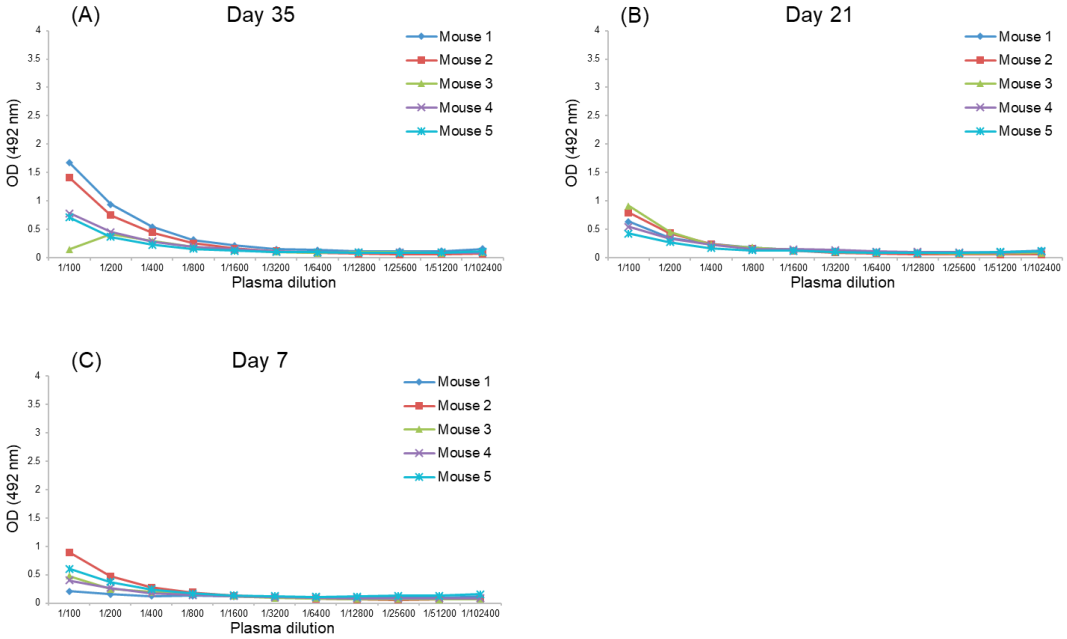


Fig. S20. ELISA of IgG antibody induced by V2 specific for BSA-triSTn on day A) 35, B) 21, or C) 7 of the immunization schedule.

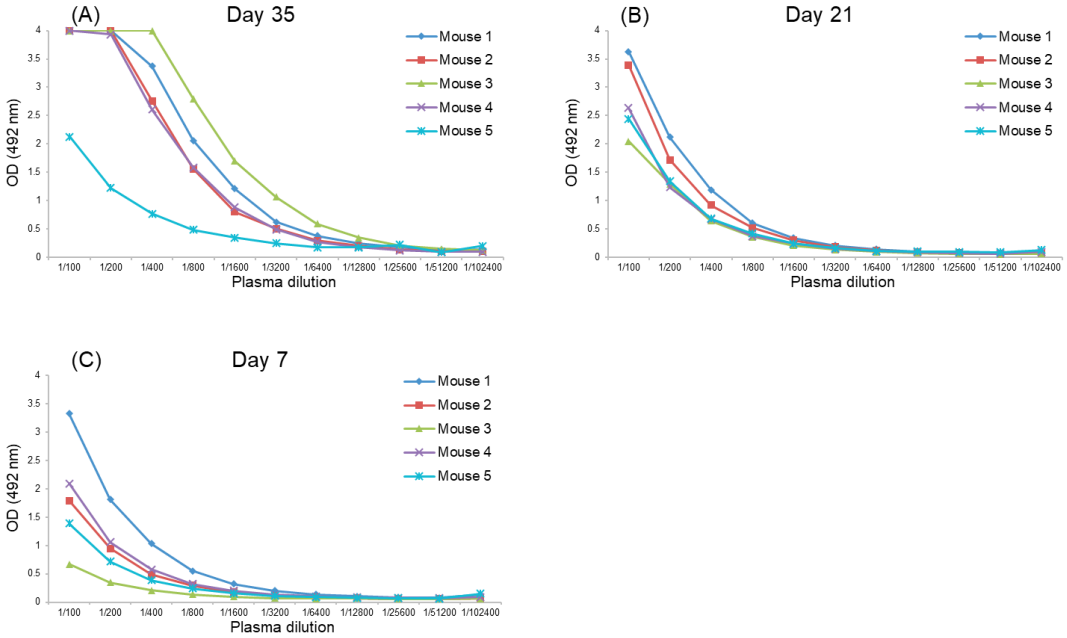


Fig. S21. ELISA of IgM antibody induced by V1 specific for BSA-STn on day A) 35, B) 21, or C) 7 of the immunization schedule.

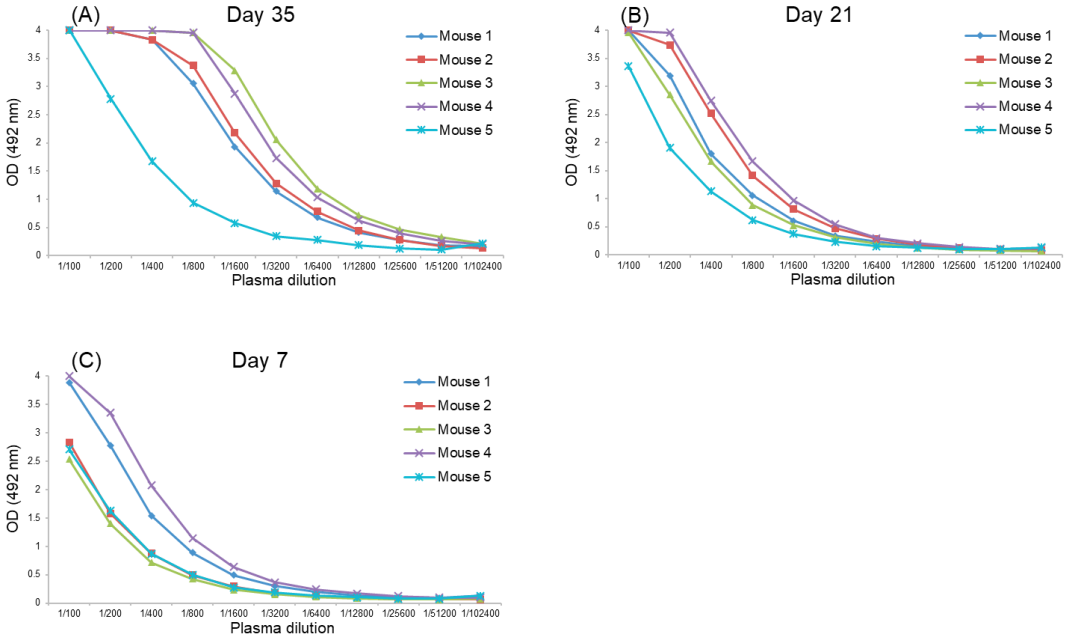


Fig. S22. ELISA of IgM antibody induced by V1 specific for BSA-triSTn on day A) 35, B) 21, or C) 7 of the immunization schedule.

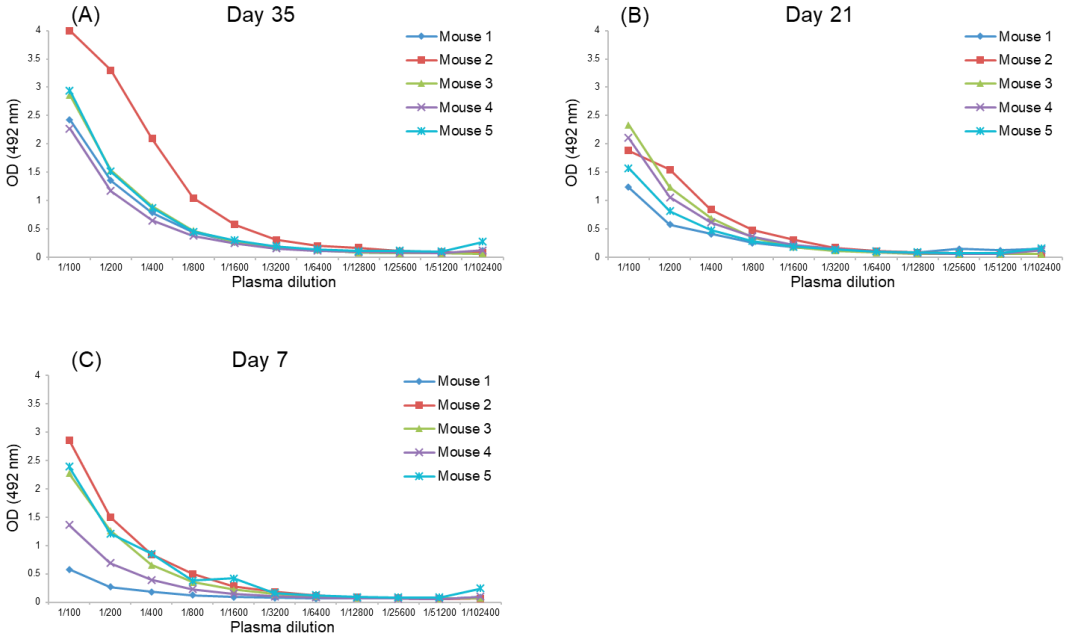


Fig. S23. ELISA of IgM antibody induced by V2 specific for BSA-STn on day A) 35, B) 21, or C) 7 of the immunization schedule.

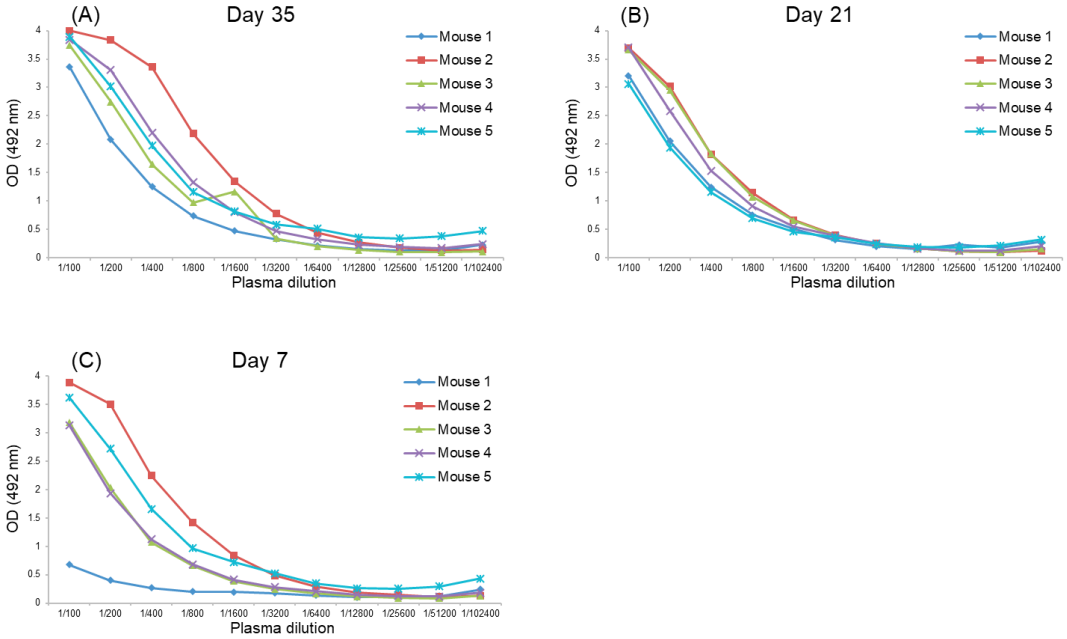


Fig. S24. ELISA of IgM antibody induced by V2 specific for BSA-triSTn on day A) 35, B) 21, or C) 7 of the immunization schedule.

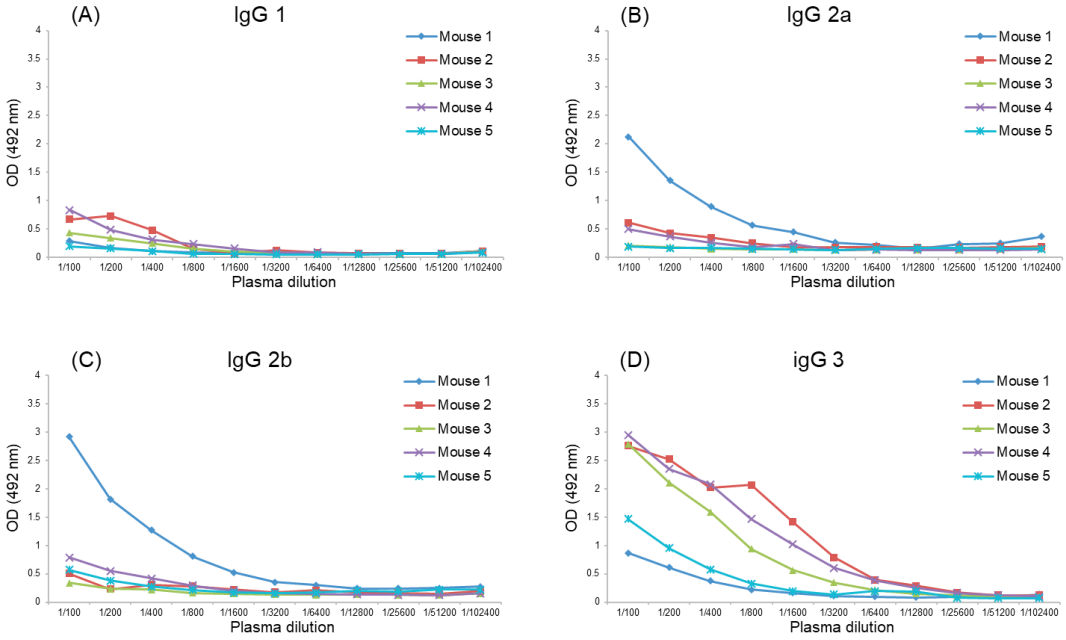


Fig. S25. ELISA of BSA-triSTn-specific A) IgG 1, B) IgG 2a, C) IgG 2b, and D) IgG 3 antibody induced by V1 on day 49 of the immunization schedule.

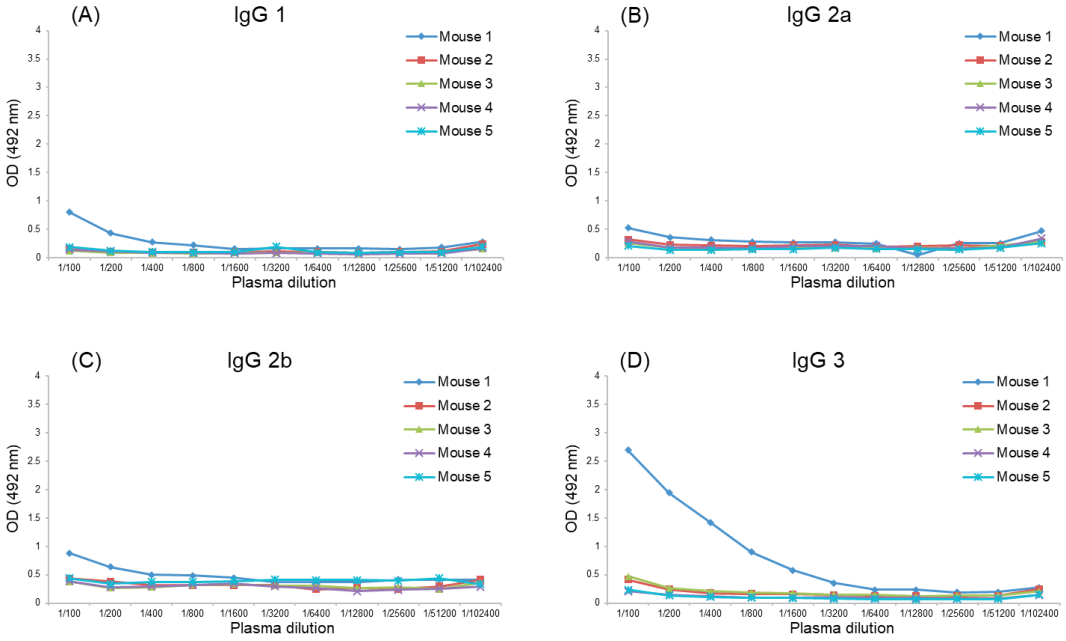


Fig. S26. ELISA of BSA-triSTn-specific A) IgG 1, B) IgG 2a, C) IgG 2b, and D) IgG 3 antibody induced by V2 on day 49 of the immunization schedule.

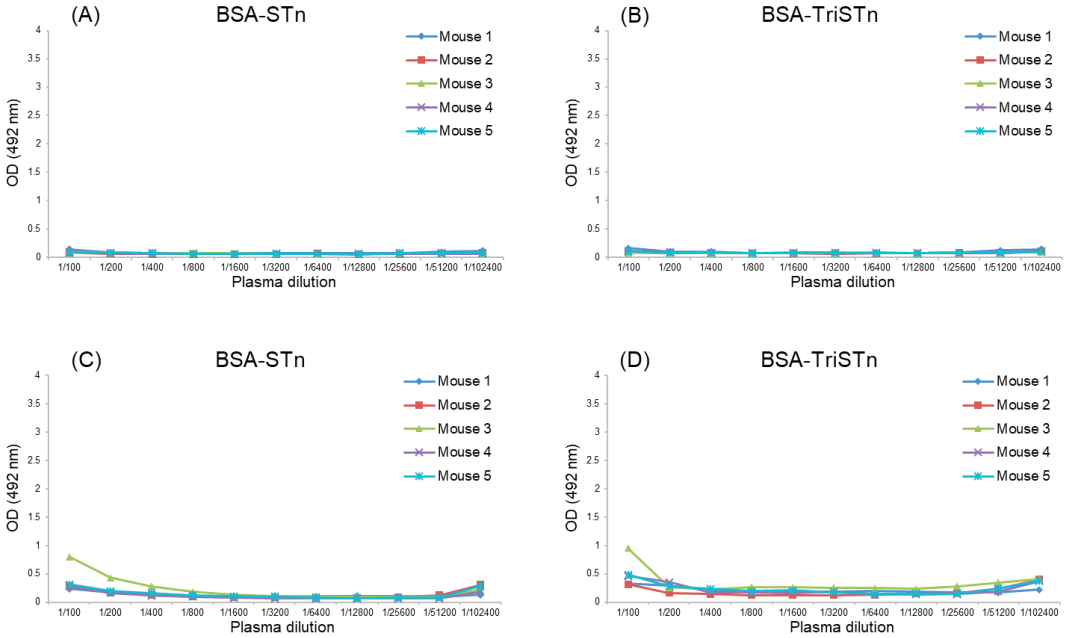


Fig. S27. ELISA of IgG antibody specific for A) BSA-STn or B) BSA-TriSTn on day 0 of the immunization schedule. ELISA of IgM antibody specific for C) BSA-STn or D) BSA-TriSTn on day 0 of the immunization schedule.

5. Protocol and data of flow cytometry

Protocol for flow cytometry

The spleen of each mouse was collected on day 49, filtered with mesh (77 μm), and centrifuged (800×g, 5 min). The precipitation was suspended with RBC Lysis Buffer (10 mL) and centrifuged (800×g, 5 min). The spleen cells were obtained as a precipitation, suspended in CELLBANKER, and stored in liq. N_2 before use. After the spleen cells were washed with PBS buffer two times, the cells (3×10^5) were stained with the respective marker antibodies (1:9) 15 min at 4 ºC. After washing with PBS buffer, the cells were analyzed using BD FACS Verse™ (BD Biosciences). Each gate was defined as a polygon in the panel and a line in the histogram. Mean fluorescence intensity (MFI) was shown in the upper right of each histogram.

<The following antibodies were used for cell staining>

Antibody	Iso type	Company	Clone	Cat.No.
CD3e FITC	Hamster IgG	TONBO biosciences	145-2C11	35-0031-U100
CD4 PE	Rat IgG2b k	eBioscience	GK1.5	12-0041-83
CD8a APC	Rat IgG2a k	eBioscience	53-6.7	17-0081-81
CD11b PE	Rat IgG2b k	BD Biosciences Pharmingen	M1/70	557397
CD11c PE-cy7	Hamster IgG	TONBO biosciences	N418	60-0114-100U
CD19 APC	Rat IgG2a k	TONBO biosciences	1D3	20-0193-100U
CD80 FITC	Hamster IgG2 k	BD Biosciences Pharmingen	16-10A1	553768
CD86 PE	Rat IgG2a k	BD Biosciences Pharmingen	GL1	553692
CD138 PE	Rat IgG2a k	BD Biosciences Pharmingen	281-2	09345B

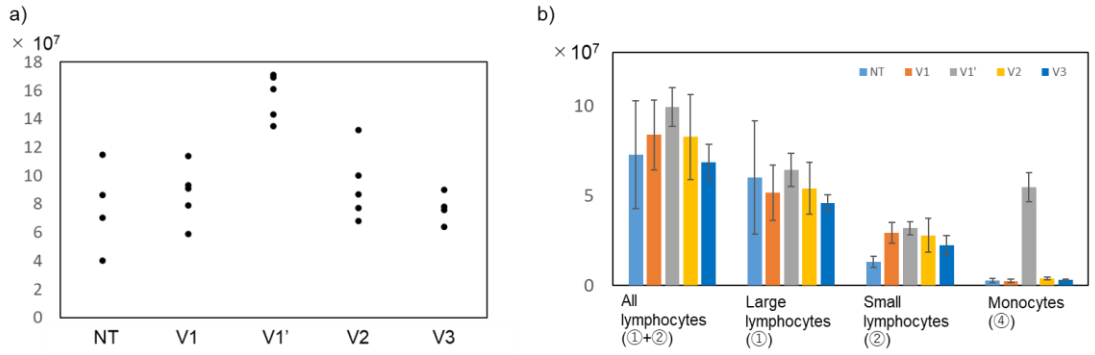
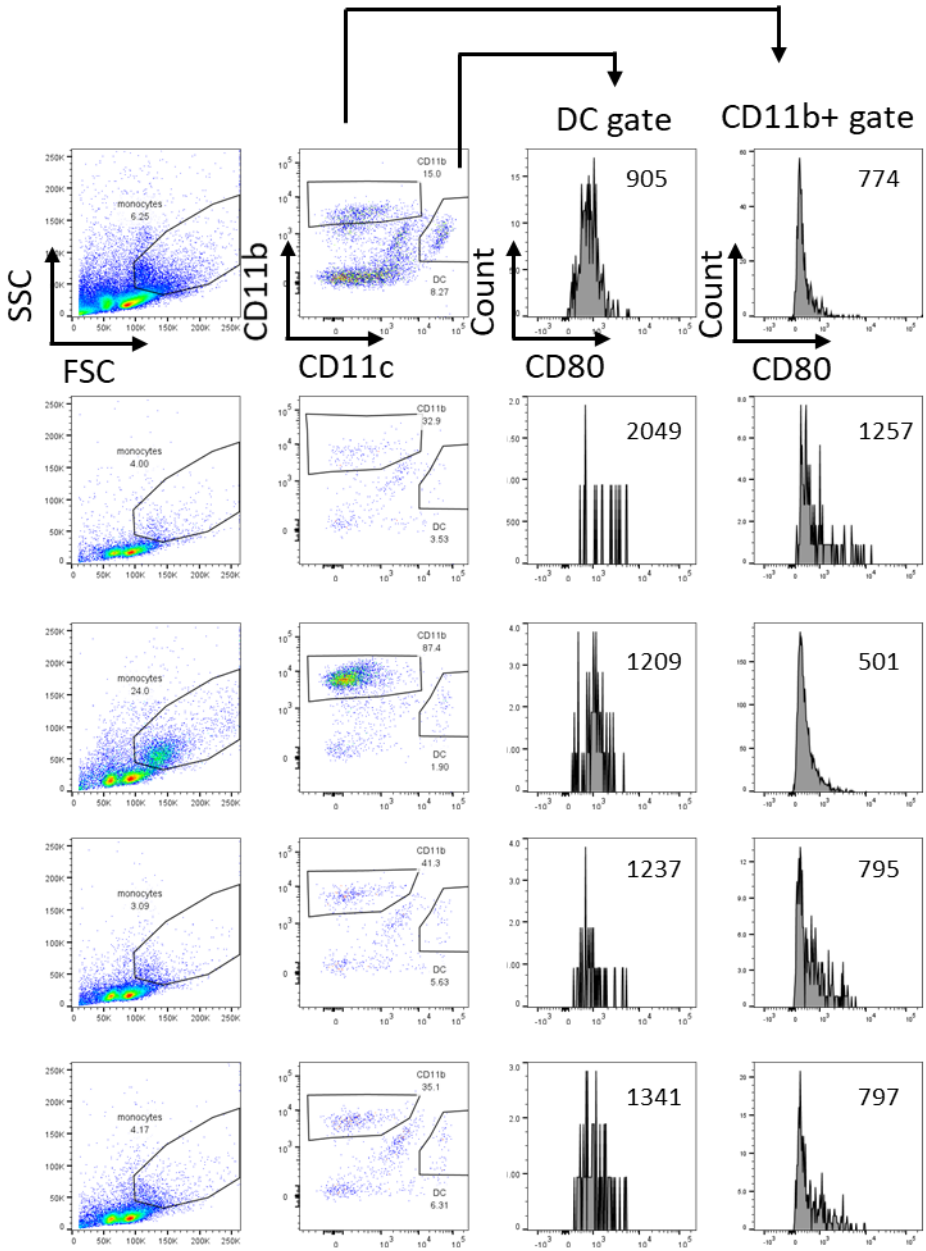


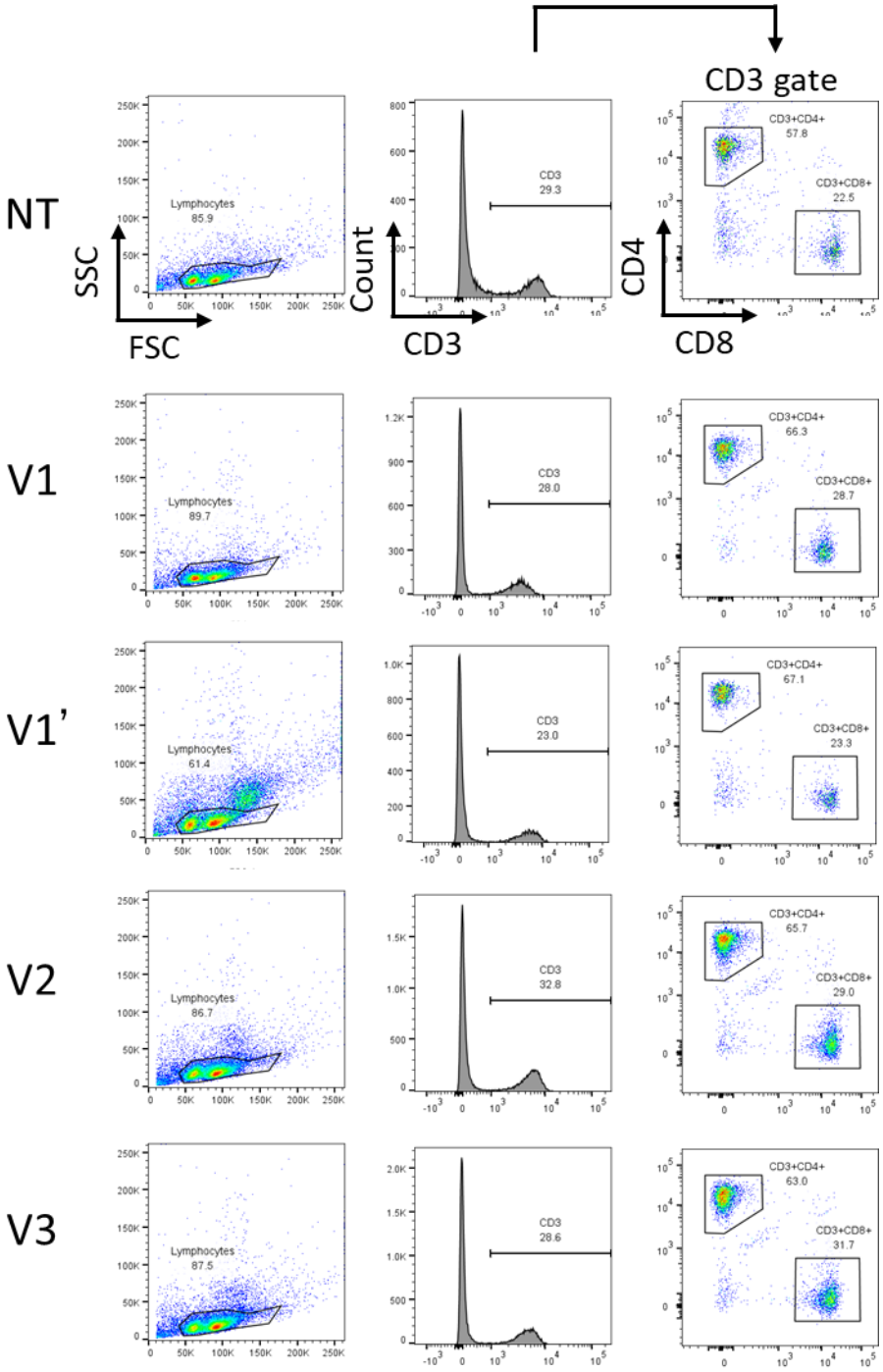
Fig. S28. Number of spleen cells on day 49 of the immunization schedule. a) Total number of spleen cells. b) Number of spleen cells classified to each cell type (lymphocyte, large lymphocyte, small lymphocyte, and monocyte).

6. Gate of flow cytometry analysis

<Dendritic cell analysis, CD11b+ cell analysis>



<T cell analysis>



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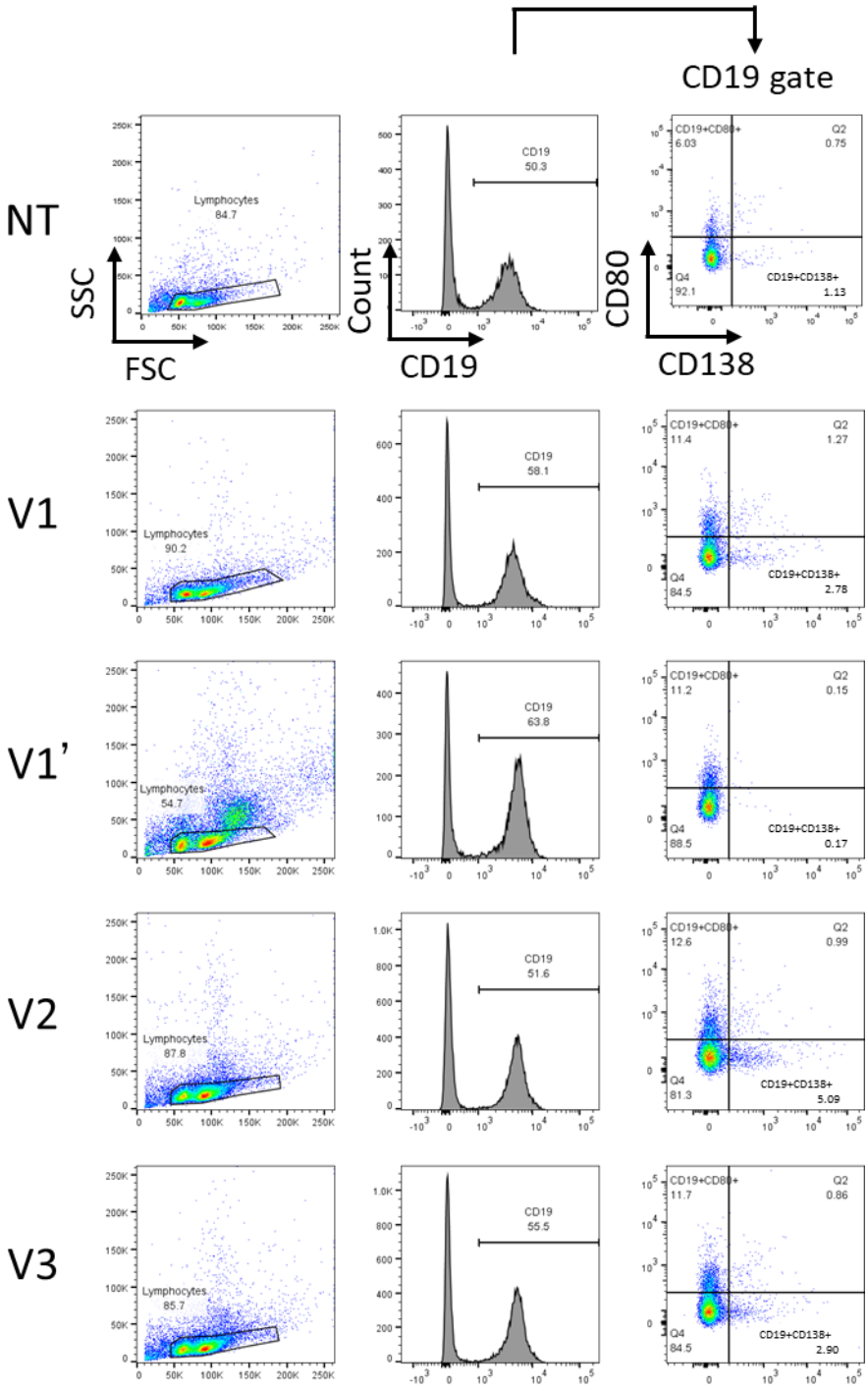
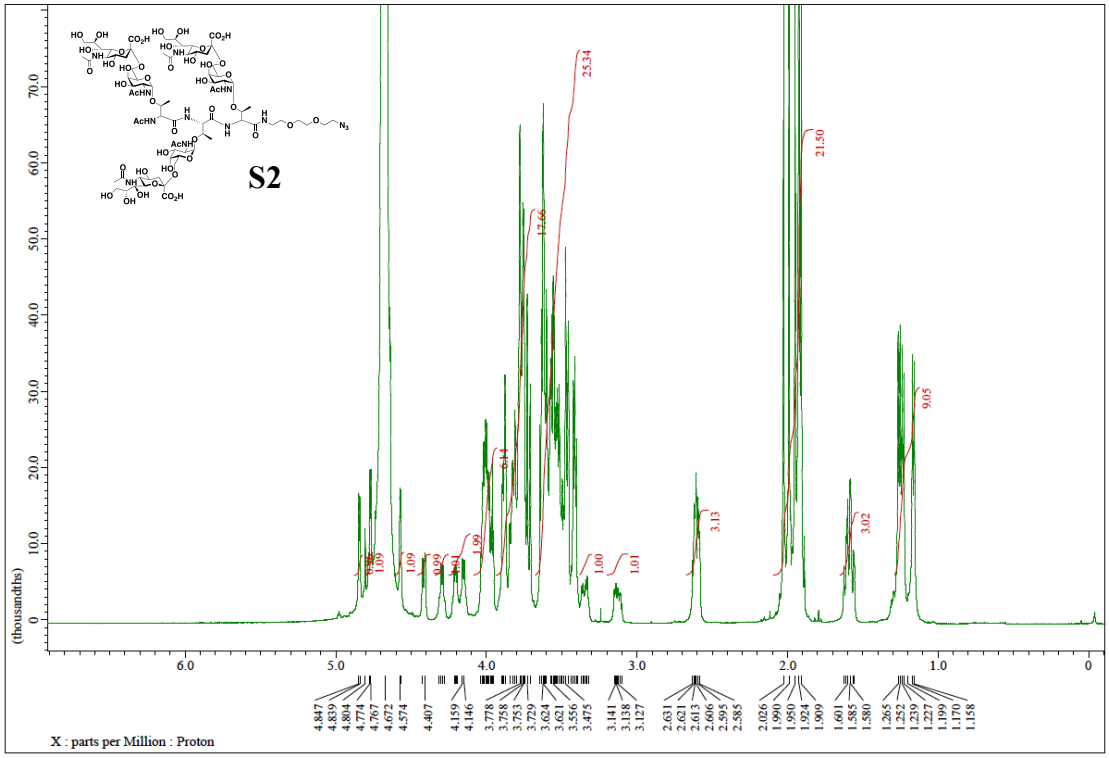


7. Reference

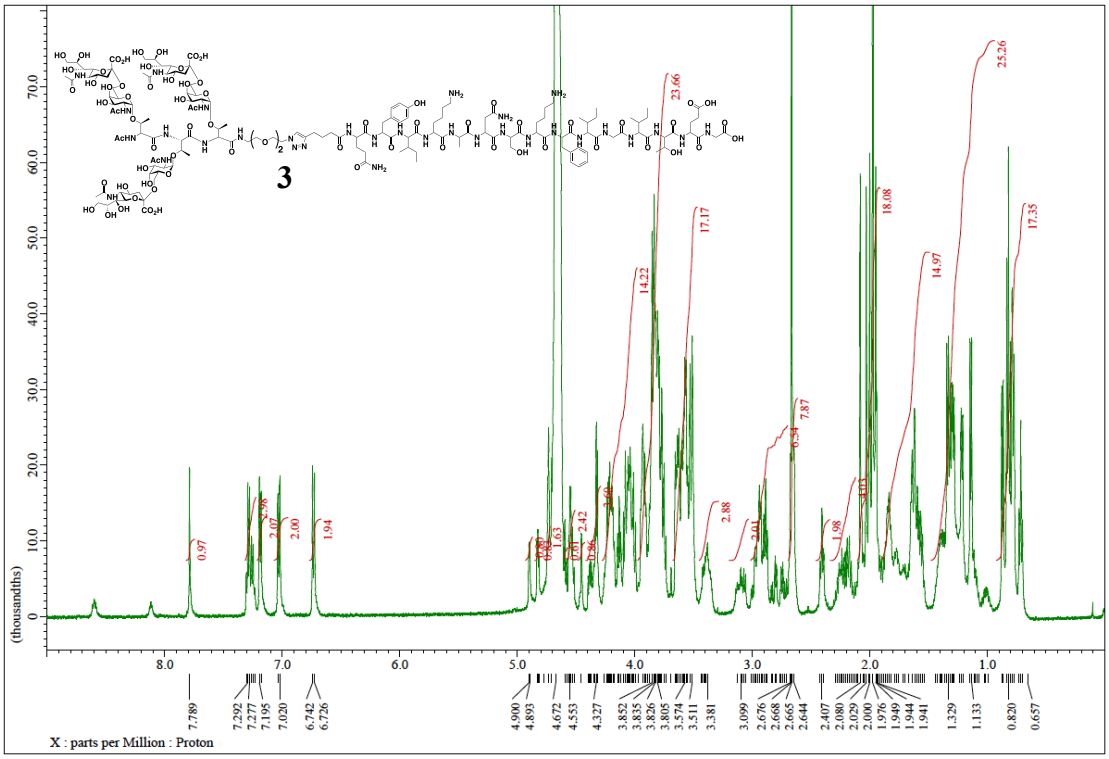
1) T.-C. Chang, Y. Manabe, Y. Fujimoto, S. Ohshima, Y. Kametani, K. Kabayama, Y. Nimura, C.-C. Lin and K. Fukase, *Angew. Chem. Int. Ed.*, 2018, **57**, 8219-8224.

8. ^{1}H and ^{13}C NMR spectra

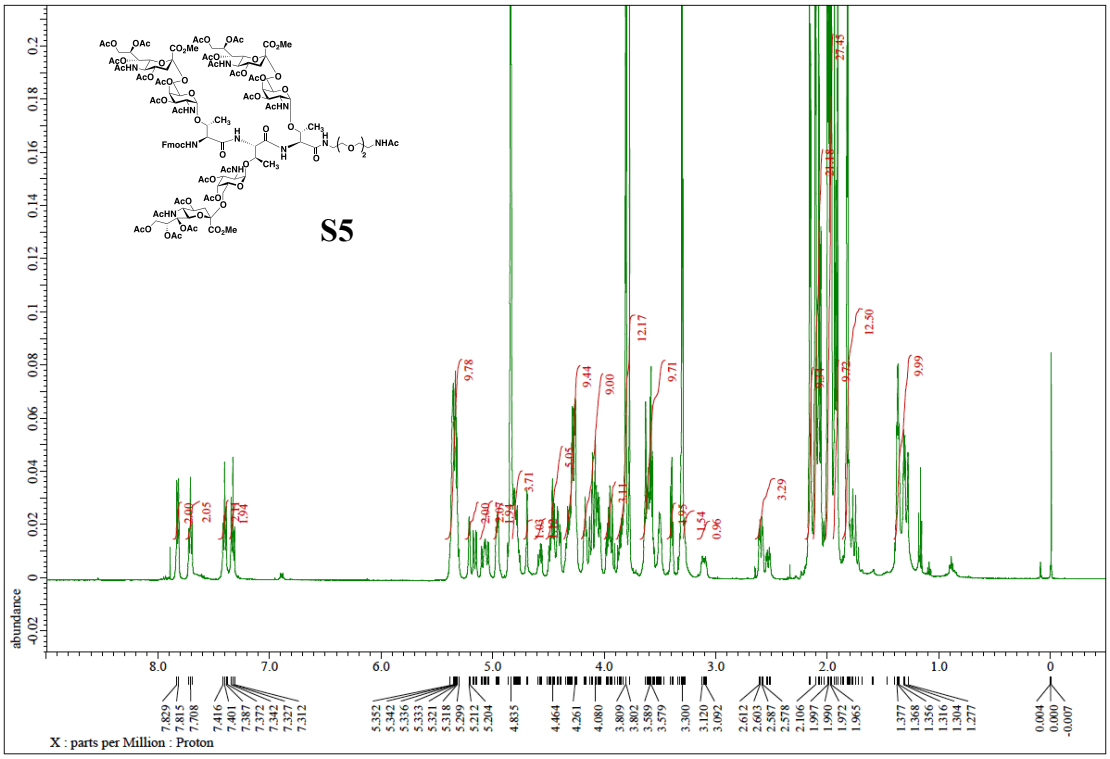
^{1}H NMR spectrum for **S2** (D_2O, 500 MHz)



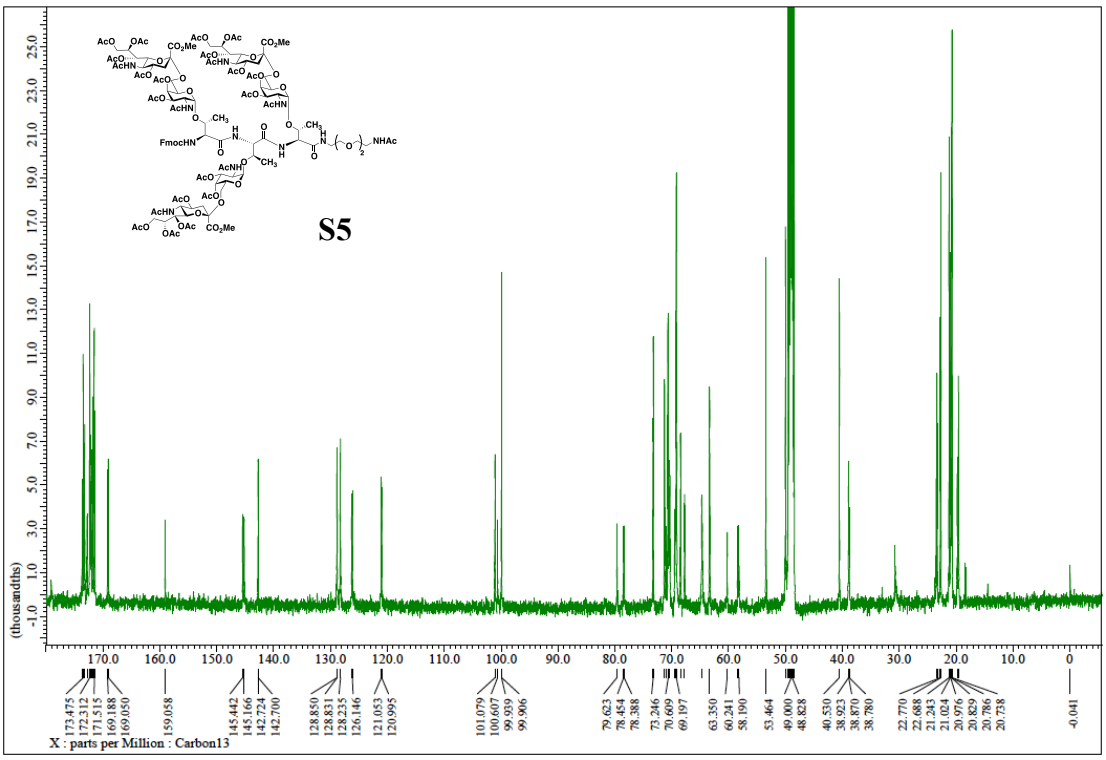
^{1}H NMR spectrum for **3** (D_2O, 500 MHz)



^{1}H NMR spectrum for **S5** (CD_3OD, 500 MHz)



^{13}C NMR spectrum for **S5** (CD_3OD, 500 MHz)



^{1}H NMR spectrum for **S8** (D_2O, 500 MHz)