Supporting Information

Structure Guided Design of Bacteriophage Qβ Mutants as Next Generation Carriers for Conjugate Vaccines

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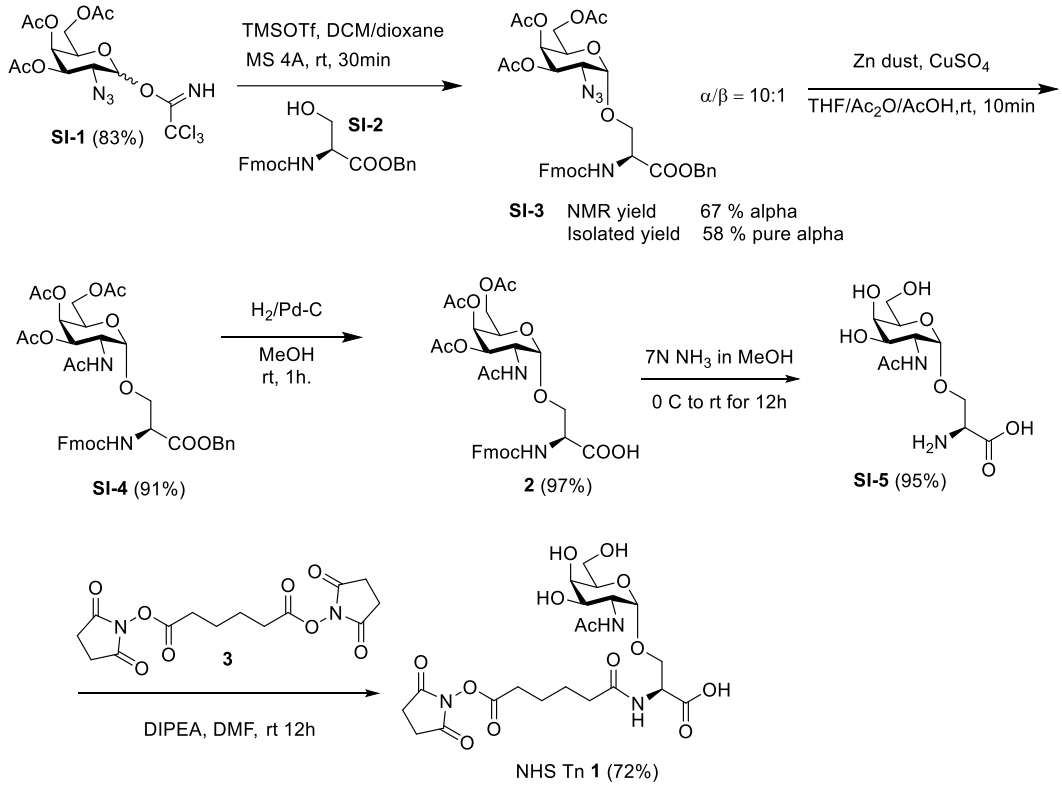
Table of Contents

Synthesis of Tn1

All chemicals were reagent grade and were used as received from the manufacturer, unless otherwise noted. Solvents were dried using a solvent purification system. Glycosylation reactions were performed with 4Å molecular sieves that were flame dried under high vacuum. Reactions were visualized by UV light (254 nm) and by staining with either $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6MO_7O_{24}$*$4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL) or 5% H_2SO_4 in EtOH. Flash chromatography was performed on silica gel 601 (230-400 Mesh). NMR spectra were recorded on an Agilent-500M spectrometer and processed by MestReNova version 10.0.2.



Scheme S1: Synthesis of NHS Tn **1**.

Synthesis procedure

*N-(Fluoren-9-ylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-serine benzyl ester (**SI-3**)*[1]*:*

Trichloroacetimidate **SI-1**[2] (3.01 g, 6.33 mmol) and *N*-Fmoc-*O*-Bn-Serine **SI-2**[3] (2.2 g, 5.28 mmol) were mixed in the reaction flask with freshly activated molecular sieves 4A (10 g) under nitrogen gas. Anhydrous dichloromethane (DCM):1,4-dioxane (1:1, 60 mL) were added to dissolve the mixture, and the solution was stirred at rt for 30 min. TMSOTf (0.297 mL, 1.925 mmol) was added dropwise into the reaction. The reaction was stirred at rt for an hour and checked by TLC. If there was some starting material **SI-2** left, 0.1 more eq. of TMSOTf was added and the reaction was allowed to proceed for another hour. Upon completion, diisopropylethylamine (DIPEA) was added to quench the reaction. The reaction was diluted with DCM and washed with

0.1 M HCl and then water. The organic layer was dried over Na_2SO_4 followed by concentration on a rotary evaporator. The crude product was purified by column chromatography (silica gel; 3:1 EtOAc:hexanes) to yield **SI-3** (pure alpha anomer) (2.23 g, 58%). Spectral analysis of the product compared with reported literature[1] confirmed the identity of the product. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.76 (dt, *J* = 7.7, 0.9 Hz, 2H), 7.66 – 7.59 (m, 2H), 7.44 – 7.28 (m, 9H), 6.00 (d, *J* = 8.1 Hz, 1H), 5.40 (dd, *J* = 3.4, 1.2 Hz, 1H), 5.31 – 5.19 (m, 3H), 4.87 (d, *J* = 3.6 Hz, 1H), 4.62 (dt, *J* = 8.2, 3.1 Hz, 1H), 4.45 – 4.36 (m, 2H), 4.24 (t, *J* = 7.2 Hz, 1H), 4.17 (dd, *J* = 10.9, 3.2 Hz, 1H), 4.10 – 3.94 (m, 4H), 3.59 (dd, *J* = 11.2, 3.6 Hz, 1H), 2.15 (s, 3H), 2.07 (s, 3H), 1.96 (s, 3H).

*N-(Fluoren-9-ylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine benzyl ester (**SI-4**):*[1]

The synthetic procedure was modified from reported literature.[1] Compound **SI-3** (2.41 g, 3.30 mmol) was dissolved in 3:2:1 of THF:Ac_2O:AcOH (60 mL). Zinc dust (2.72 g, 41.23 mmol) was added followed by saturated aq. $CuSO_4$ (5 mL) to activate zinc. The reaction was stirred at rt for half an hour. After completion as monitored by TLC, the zinc dust was removed by filtering the reaction mixture through Celite®. The filtrate was coevaporated with toluene to concentrate the crude product. The crude product was purified by column chromatography (silica gel; 1:1 EtOAc:hexanes) to yield **SI-4** (2.24 g, 91%). Spectral analysis of the product compared with reported literature confirmed the identity of the product. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.43 – 7.28 (m, 9H), 5.88 (d, *J* = 8.3 Hz, 1H), 5.58 (d, *J* = 9.5 Hz, 1H), 5.31 (d, *J* = 3.2 Hz, 1H), 5.20 (q, *J* = 12.1 Hz, 2H), 5.04 (dd, *J* = 11.4, 3.2 Hz, 1H), 4.78 (d, *J* = 3.7 Hz, 1H), 4.66 – 4.48 (m, 2H), 4.43 (d, *J* = 7.1 Hz, 2H), 4.23 (t, *J* = 7.1 Hz, 1H), 4.16 – 3.89 (m, 5H), 2.16 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H).

*N-(Fluoren-9-ylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (**2**):*[1]

The synthesis procedure was as reported.[1] The reaction yielded the product **2** (0.88 g, 98%). Spectral analysis of the product compared with reported literature[1] confirmed the identity of the product.

*O-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (**SI-5**):*

The synthetic procedure was adapted from reported literature.[1] To compound **2** (100 mg, 0.152 mmol) under N_2 at 0 °C was added 7N ammonia in methanol (5 mL). The reaction was warmed up to rt overnight. Upon completion, the solvent was evaporated by flowing N_2 gas. The crude reaction mixture was dissolved in MeOH and then precipitated in EtOAc. The filtrate was dried to yield **SI-5** (43.4 mg, 92 %). ^{1}H NMR (500 MHz, Methanol-d_4) δ 4.81 (d, *J* = 3.7 Hz, 1H), 4.31 (dd, *J* = 10.9, 3.6 Hz, 1H), 4.06 (bs, 1H), 3.92 – 3.65 (m, 7H), 2.01 (s, 3H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 172.4, 99.0, 71.9, 69.6, 69.2, 61.6, 50.2, 21.4. MS (ESI) m/z calcd for $C_{11}H_{20}N_2NaO_8$ $[M+Na]^+$ 331.1112, found 331.1133.

N-(N-Hydroxysuccinimidyl adipoyl)-O-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (NHS Tn **1**)*:*

To diNHS ester adipate **3** (5 eq.) in anhydrous DMF (0.5 mL) was added to **SI-5** (40 mg, 0.13 mmol) dissolved in DMF (0.5 mL). DIPEA (1 eq.) was added in the reaction mixture. The reaction was left stirred for 2-3 h. Upon completion, DMF was evaporated under vacuum until

dryness. The crude product was precipitated in EtOAc twice and then washed with 10% MeOH in EtOAc 3-5 times to remove the excess diNHS-linker **3**. The final precipitate was dried under vacuum to yield NHS Tn **1** (50 mg, 72%). ^{1}H NMR (500 MHz, Methanol-d_4) δ 4.83 (d, J = 3.7 Hz, 1H), 4.65 (t, J = 4.0 Hz, 1H), 4.25 (dd, J = 11.0, 3.7 Hz, 1H), 3.93 – 3.62 (m, 7H), 2.83 (s, 4H), 2.73 – 2.62 (m, 2H), 2.35 (t, J = 6.8 Hz, 2H), 2.00 (s, 3H), 1.83 – 1.73 (m, 4H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 174.5, 172.9, 170.9, 169.2, 98.9, 71.9, 69.3, 68.7, 68.5, 61.4, 50.4, 34.8, 30.2, 25.5, 24.9, 24.2, 21.8. MS (ESI) m/z calcd for $C_{21}H_{32}N_3O_{13}$ $[M+H]^+$ 534.1930, found 534.1951.

Synthesis and characterization of wtQβ or mQβ conjugates[4]

VLP wtQβ or mQβ (13.2 mg, 5.1 nmol particle, 0.9 μmol subunit, 3.6 μmol reactive amines for wtQβ) suspended in potassium phosphate buffer (0.1 M, pH=7, 5.5 mL) was added into a 15mL falcon tube. A solution of NHS Tn **1** in DMSO (20 mg/mL in 0.35 mL DMSO, 0.017 mmol, 4.7 eq. to each reactive amine) was added into the reaction tube. The reaction mixture was placed on a rotating mixer at room temperature overnight. The reaction was diluted with 0.1 M PBS pH=7 to a total volume of 50 mL. The VLP conjugates were purified by filtration through Millipore 100k MWCO centrifugal filter tube, and washed thoroughly with the PBS buffer. The flow through after every buffer exchange cycle was checked by the anthrone reagent to monitor successful removal of unconjugated Tn antigen. The final amounts of wtQβ or mQβ recovered were determined by the Bradford assay using BSA as a standard. The average number of conjugated Tn on each viral capsid subunit was estimated from the intensity of peaks in the deconvoluted mass spectra from MS analysis. The loading levels of NHAcGD2 and SARS-CoV-2 peptide antigens per capsid were determined in a similar manner. Representative MS results are shown in **Figure S1**.

Procedure for size exclusion chromatography (SEC)

SEC analysis and purification of the various VLP Qβ were performed on an AKTApure 25L system, equipped with Superose 6 Increase 10/300 GL column. Potassium phosphate buffer (pH=7, 0.1 M) was used as the eluent with a flow rate of 0.5 mL/min at 4 °C. 0.5 mL of sample was injected. The sample was eluted with 1.5 column volume and the fractions were collected every 1mL. The capsid protein was detected with a UV detector at the wavelength of 280nm.

Synthesis and characterization of CRM197-Tn and Tetanus Toxoid-Tn (TT-Tn) conjugate

CRM197 (2 mg) or tetanus toxoid (2mg, both from a 5 mg/mL stock solution in 0.1 M HEPES buffer, pH 8.0; Fina Biosolutions) was mixed with 1.25 mg or 1.45 mg of NHS Tn **1** respectively (from 20 mg/mL stock solution in DMSO) in a sterile 1.5 mL microcentrifuge tube. The final volume of the mixture was brought to 1 mL with 0.1 M HEPES buffer, pH 8.0. The tube was placed in a thermomixer and the conjugation was allowed to proceed at 37 °C for 1 hour with constant shaking at 300 RPM. The mixture was transferred into a 15 mL centrifuge tube and diluted with sterile 1X PBS, pH 7.4 to a total volume of 5 mL, followed by sterile filtration through 0.22 μm PVDF syringe filter. The mixture was purified using a 4 mL, 10 kDa Amicon ultra centrifugal filter tube (MilliporeSIgma) by buffer exchange with fresh 1X PBS buffer (3000 xg, 10 min, 4 °C) to remove unreacted Tn antigen. The flow through after every buffer exchange cycle was checked by the anthrone reagent to monitor successful removal of unconjugated Tn antigen. The final amounts of CRM197-Tn and TT-Tn recovered were determined by the Bradford assay using BSA as a standard and nanodrop by measuring absorbance at 280 nm. Successful conjugation of Tn to protein carriers was determined by SDS-PAGE under reduced conditions, while quantification of the number of Tn per protein was determined by MALDI-TOF-MS. The quantification established that there were on average 10 copies of Tn per TT and 20 copies of Tn per CRM197.

Synthesis and characterization of KLH-Tn conjugate

KLH lyophilized powered (MilliporeSigma) was reconstituted with sterile water to a final concentration of 5 mg/mL. The commercial lyophilized powder contains sucrose. Thus, it was necessary to remove the sucrose as it could potentially be a contaminant during downstream process. The removal was achieved by buffer exchange with 0.1 M HEPES buffer, pH 8.0 using 100 kDa Amicon ultra centrifugal filter tube. The flow through after every buffer exchange cycle was monitored by anthrone reagent to ensure complete removal of sucrose. 80% of KLH was recovered as determined by the Bradford assay against BSA standard. 2 mg of KLH (from a 4 mg/mL stock solution in 0.1 M HEPES buffer, pH 8.0) was mixed with 0.65 mg NHS Tn **1** (from 20 mg/mL stock solution in DMSO) in a sterile 1.5 microcentrifuge tube. The final volume of the mixture was brought to 1 mL with 0.1 M HEPES buffer, pH 8.0. The usage of HEPES buffer gave higher conjugation efficiency as compared to potassium phosphate buffer at pH7.0. The tube was placed in a thermomixer and the conjugation was allowed to proceed at 37 ^{o}C for 1 hour with constant shaking at 300 RPM. The mixture was transferred into a 15 mL centrifuge tube and diluted with sterile 1X PBS, pH 7.4 to a total volume of 5 mL, followed by sterile filtration through 0.22 µm PVDF syringe filter. The buffer exchange purification protocols described above for CRM197-Tn and TT-Tn were repeated except that 100 kDa Amicon ultra centrifugal filter tube was used instead of the 10 kDa filter tube. Similarly, the final recovered KLH-Tn was determined by Bradford assay against BSA as a standard. Quantification of the number of Tn conjugated to KLH was determined by the Anthrone assay following reported protocols[5] using GalNAc as the standard. Unconjugated KLH was used as a control to correct the background since KLH is a glycoprotein. The quantification established that there were about 3,000 copies of Tn per KLH.

MS analysis of mQβ-Tn, CRM197-Tn and TT-Tn conjugates

20 µL of either mQβ-Tn, CRM197-Tn and TT-Tn and the unconjugated version of mQβ, CRM197 and TT in a 0.5 mL microcentrifuge tube (at 1 mg/mL, containing DTT at a final concentration of 50 mM) was reduced by heating in a water bath at 95 ^{o}C for 5 min. After allowing contents to cool to room temperature, the reduced protein-Tn conjugates were desalted using ZipTip C4 pipette tips (Millipore-Sigma). The C4 pipette tips were conditioned prior to desalting of protein mixture following manufacturer's protocols. The bound protein mixture was eluted into a clean microcentrifuge tube with 5 µL of 50/50/0.1, acetonitrile/water/trifluoroacetic acid. 1 µL of the eluted mixture was mixed with 1 µL of sinapic acid (10 mg/mL in 50/50/0.1, acetonitrile/water/trifluoroacetic acid) and spotted on a MALDI plate. The plate was allowed to air-dry at room temperature followed by analysis on a MALDI-TOF mass spectrometry (Shimadzu Biotech Axima).

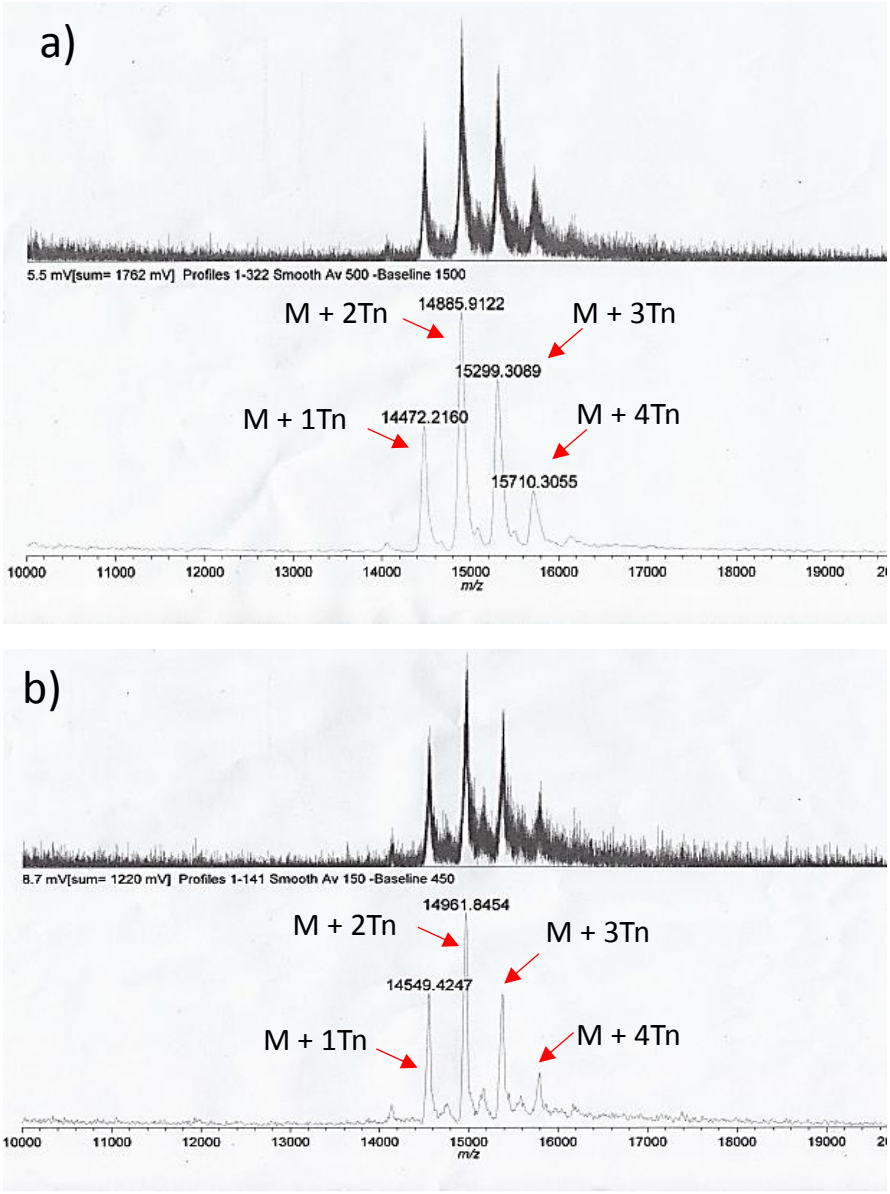


Figure S1. MALDI-TOF-MS spectra of a) wtQβ-Tn and b) mQβ-A38K/A40C/D102C-Tn conjugates. M refers to the corresponding bacteriophage Qβ coat protein monomer. Based on the intensities of the MS peaks, for both wtQβ and mQβ-A38K/A40C/D102C, it is calculated that there are on average 370 copies of Tn per capsid as the capsid is made up of 180 copies of the coat protein monomer.

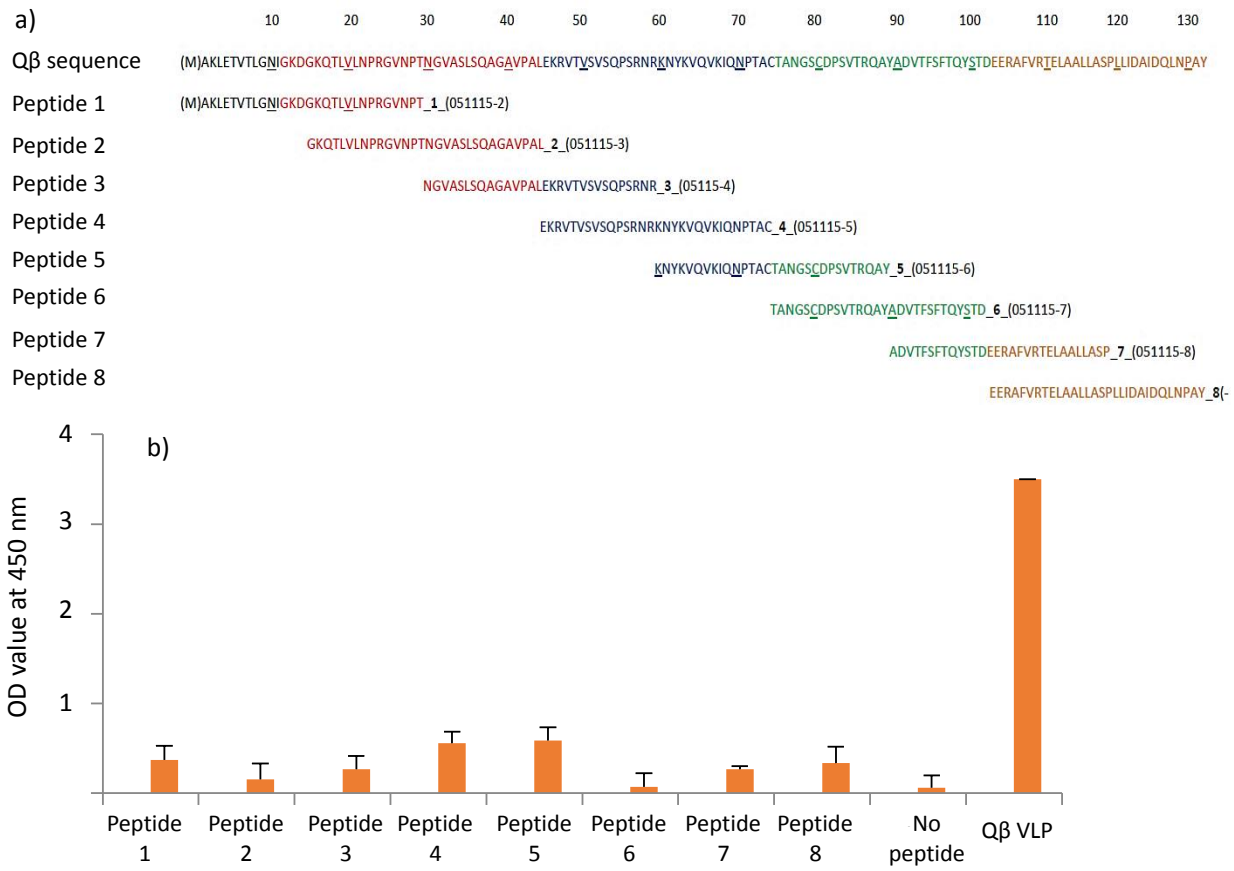


Figure S2. a) The sequences of a panel of 8 peptides covering the full sequence of bacteriophage Qβ coat protein. b) ELISA analysis of sera from Qβ immunized mice (1:64,000 dilution) showed that there was little recognition of the linear peptides of bacteriophage Qβ coat protein as compared to the full Qβ capsid. This suggests that the B cell epitopes of bacteriophage Qβ coat protein most likely are conformational.

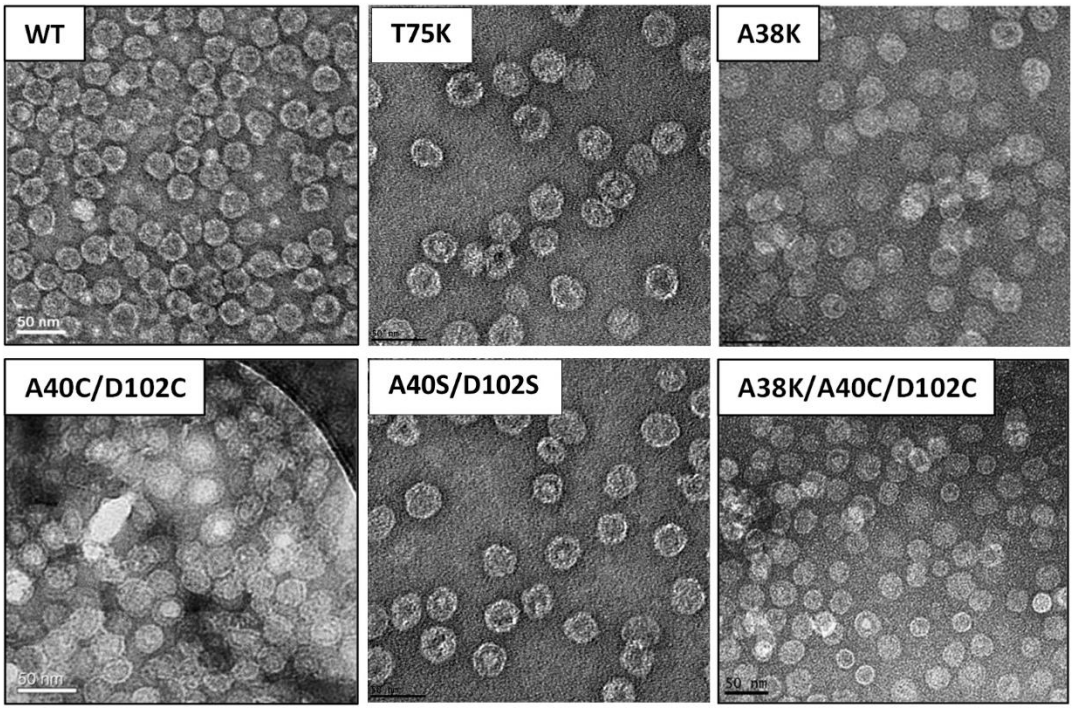


Figure S3. TEM images of wtQβ and various mQβs. The scale bars are 50 nm.

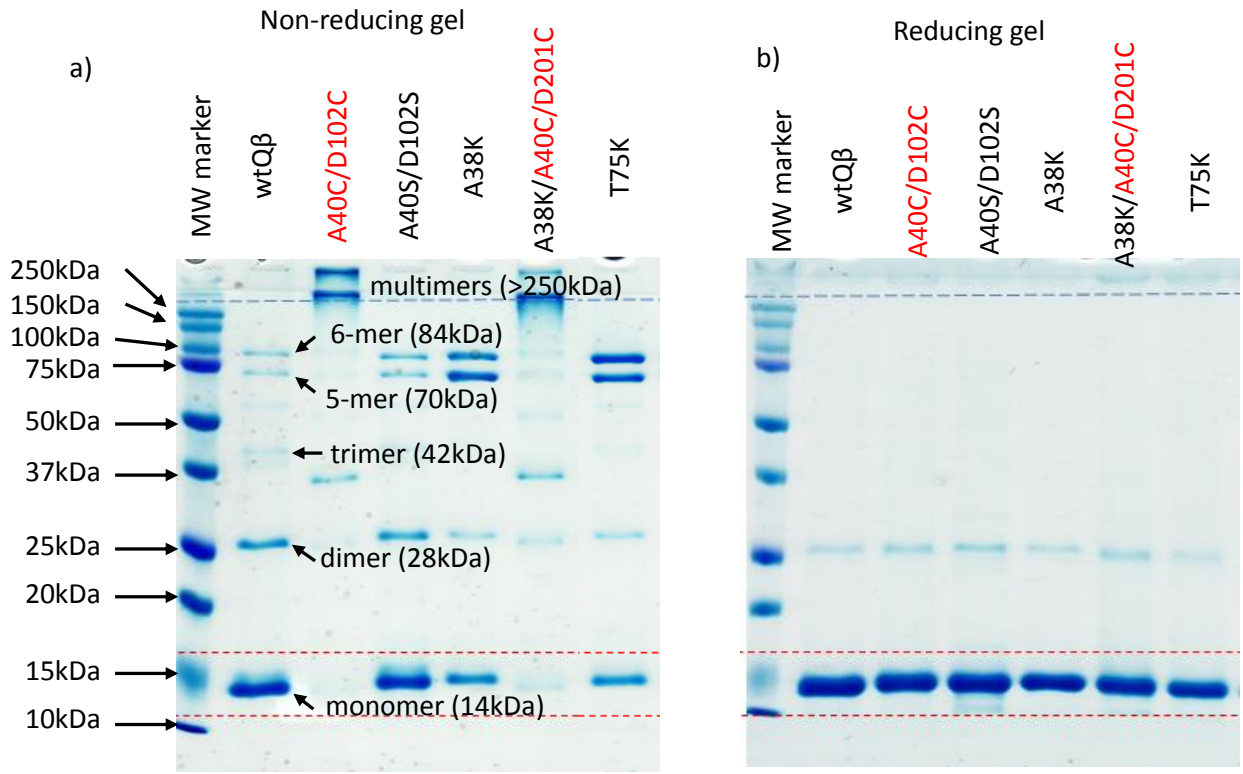


Figure S4: a) SDS-PAGE of the Qβ capsids under a) non-reducing condition and b) reducing condition upon staining with Coomassie Blue Stain. The low intensities of the bands at 14 kDa for the lanes of Qβ containing A40C/D102C on the non-reducing gel as compared to those on the reducing gel support the formation of the A40C/D102C disulfide bond.

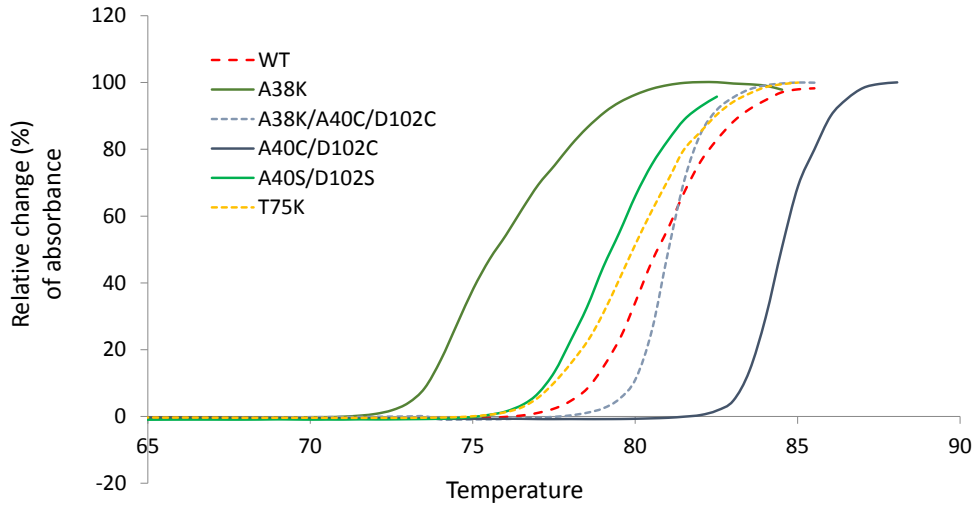


Figure S5. Relative changes of UV absorbance values (λ = 310 nm) of various Qβ VLPs with increasing temperatures. The introduction of A40C/D102C mutations increased the thermal stability of the capsid.

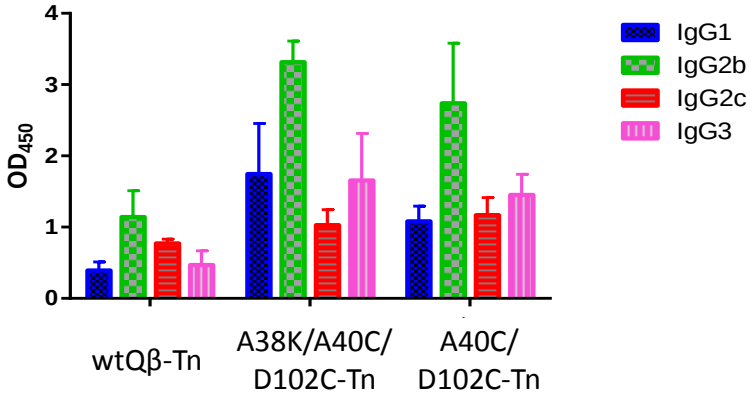


Figure S6. Similar subtype profiles of IgG antibodies (IgG1, IgG2b, IgG2c and IgG3) were elicited by wtQβ-Tn, mQβ(A38K/A40C/D102C)-Tn and mQβ(A40C/D102C)-Tn as determined by OD_{450} values at 1/819,200 serum dilution.

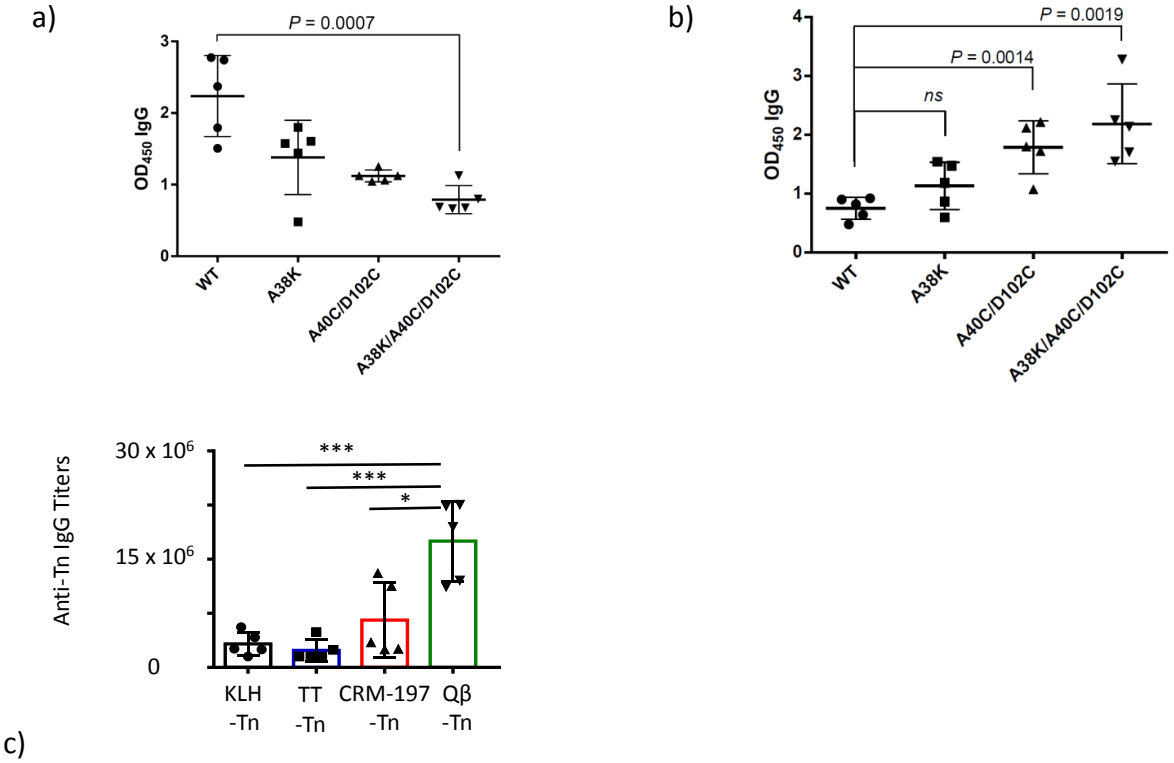


Figure S7. ELISA results of post-immunized sera (day 35) from groups of mice (*n*=5) vaccinated with wtQβ-Tn, A38K-Tn, A40C/D102C-Tn, and A38K/A40C/D102C-Tn respectively. Correlation with anti-Tn antibody responses from Tn conjugates of A38K, A40C/D102C and A38K/A40C/D102C exhibited an interesting reverse trend of anti-carrier *vs* anti-Tn antibodies with the vaccine construct producing lower anti-carrier antibody responses while eliciting higher levels of anti-Tn IgG. a) OD_{450} of anti-carrier IgG antibodies in the post-immunized sera at 1/1,638,400 against the corresponding carrier capsids. b) OD_{450} of anti-Tn IgG antibodies in the post-immunized sera at 1/819,200 dilution against BSA-Tn. The statistical significance was determined by the student t test using GraphPad Prism. c) The titers of anti-Tn IgG antibodies in sera from mice immunized with KLH-Tn, TT-Tn, CRM-197-Tn, and mQβ-Tn respectively were determined. mQβ is a superior carrier to generate anti-Tn IgG antibody responses when compared with the common carriers including KLH, TT and CRM-197. Statistical analysis was performed using the Student t test by GraphPad Prism. * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$.

Site-directed mutagenesis of Qβ VLPs

Primers were designed following a guild line in the manual of QuikChange® Site-Directed Mutagenesis Kit. Online based software PrimerX (http://www.bioinformatics.org/primerx/) was used to generate the primer sequence, or the sequence could be designed manually in some cases. The designed primers were further analyzed for proper %GC and Tm again by Oligoanalyzer 3.1 from IDT. Inc. All primers were commercially synthesized by IDT. Inc. For PCR reaction, the reagent mixture and cycling setting are prepared by adding reagents, respectively, as followed,

Reagents	**µl**
10X buffer	5
20 ng plasmid template (from 20 ng/µl)	1
125 ng Forward primer (from 100 ng/µl)	1.25
125 ng Reverse primer (from 100 ng/µl)	1.25
dNTP mix	1
BP561-1 water	39.5
Pfu turbo	1

PCR thermocycler setting

Number of cycles	Temperature	Time
1X	95 °C	30 Sec
17X	95 °C	30 Sec
	5 °C less than T_m of the primers, or using gradian	1 Min
	68 °C	6 Min
Finish	4 °C	till done

After the PCR reaction, 1μl *Dpn*I was added to the resulting reaction mixture, which was further incubated at 37°C for 1 hour to digest the template plasmid DNA. The reaction's products were verified by 0.8% agarose gel electrophoresis with ethidium bromide as a staining reagent. The reactions were then used without purification to transform DH5α *E.coli*. The transformed DH5α E. coli cultures were plated onto a SOB agar plate with 20μg/mL Kanamycin sulfate and incubated at 37°C overnight. 4-6 colonies were selected to inoculate 6 mL SOB with 20ug/mL Kanamycin, which were incubated at 37°C overnight to amplify the *E. coli*. Mutated DNA plasmid from the bacteria cultures were extracted with QIAprep Spin Miniprep Kit (QIAGEN). The extracted plasmids were submitted to GENEWIZ for sequencing. Plasmids of the mutants that provided the correct DNA sequences with highest scores of sequencing quality were used for transformation into BL21(DE3)pLysS *E. coli* by the heat shock method, then plated on SOB agar plate as described for DH5α *E. coli*. A single colony was selected for protein expression.

Accessible surface area measurement

The Relative Accessible Surface Area (RASA) of each residue in the Qβ VLP crystal structure was calculated by measuring solvent accessible surface area in the context of the full capsid using FreeSASA[6] and expressing as a percent of the maximum theoretical surface area[7] of each residue type. RASA values for each residue represent average values of all chains in the crystal's asymmetric unit.

Table S1: Physical characteristics of wtQβ and various Qβ mutants. The retention volumes (mL) on a Superose SEC column of the Qβ VLPs are presented. The average hydrodynamic diameter, polydispersity index (PDI), and zeta potential values were obtained using Zetasizer DLS.

Qβ	**SEC elution volume (**mL**)**	**Average diameter (**nm**)**	**Polydispersity index**	**Zeta potential** (mV)
wt	11.7	28.79	0.046	-2.89
A38K	12.7	26.58	0.083	-1.43
T75K	12.0	29.02	0.096	-1.64
A40C/D102C	11.8	29.06	0.031	-1.67
A38K/A40C/D102C	12.6	27.55	0.028	-1.55
A40S/D102S	12.0	27.82	0.028	-1.37

Table S2: Primers used in the construction of mutant Qβ VLPs

Primer #	**Name**	**Sequence**
1	CP_A38K_F1	5'-GCCTCGCTTTCACAA**AAG**GGTGCAGTTCCTGCG-3'
2	CP_A38K_R1	5'-CGCAGGAACTGCACC**CTT**TTGTGAAAGCGAGGC-3'
3	CP_A38K_[A40C]_F1	5'-CCTCGCTTTCACAA**AAG**GGTTGTGTTCCTGC-3'
4	CP_A38K_[A40C]_R1	5'-GCA GGAACACAACC**CTT**TTGTGAAAGCGAGG-3'
5	CP_A40C_F1	5'-CACAAGCGGGT**TGT**GTTCCTGCGCTGG-3'
6	CP_A40C_R1	5'-CCAGCGCAGGAAC**ACA**ACCCGCTTGTG-3'
7	CP_A40S_F1	5'-CACAAGCGGGT**TCA**GTTCCTGCGCTGG-3'
8	CP_A40S_R1	5'-CCAGCGCAGGAAC**TGA**ACCCGCTTGTG-3'
9	CP_T75K_F1	5'-CCGACCGCTTGC**AAG**GCAAACGGTTC-3'
10	CP_T75K_R1	5'-GAACCGTTTGC**CTT**GCAAGCGGTCGG-3'

Enzyme-Linked Immunosorbent Assay (ELISA)

A Nunc MaxiSorp® flat-bottom 96 well plate (Sigma M5785-1CS) was coated with BSA-Tn (100 µL, 10µg/mL), the corresponding Qβ capsids (100 µL, 1µg/mL), BSA conjugates of RBD peptides (1 µg), or RBD (1 µg) in PBS pH = 7.4, overnight at 4 °C. The coated plate was then washed 4 times with PBS/0.5% Tween-20 (PBST), followed by the addition of 1% (w/v) BSA in PBS to each well and incubation at room temperature for one hour. The plate was washed again 4 times with PBST. Diluted mouse sera in 0.1% BSA/PBS (100 µl) were added in each well. The plate was incubated for two hours at 37 °C and washed. A 1:2000 diluted horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, IgG1, IgG2b, IgG2c, IgG3 or IgM antibody (Jackson ImmunoResearch Laboratory IgG #115−035−071, IgM #115−035−075) in 0.1% BSA/PBS was added to each well, respectively. The plate was incubated for one hour at 37 °C, washed, and a solution of 3,3′,5,5′-tetramethylbenzidine (TMB) (Sigma 860336) was added. Color was allowed to develop for 15 min, and then a solution of 0.5 M H_2SO_4 (50 µl) was added to stop the reaction. The optical density was measured at 450 nm using a microplate autoreader (Molecular Devices SpectraMax M3). OD450 was fitted to log10 of the dilution folds with 4 PL nonlinear regression method (GraphPad Prism, least squares). Endpoint titers was calculated to the dilution folds that gives OD450 equivalent of 0.2 absorbance units above background. All steps were performed under ambient temperature unless specified. Each experiment was repeated at least four times, and the average of the quadruplicate was used to calculate the titer.

Cell culture

Murine mammary adenocarcinoma cell line TA3Ha (kindly provided by Prof. John Hilkens, The Netherlands Cancer Institute) were isolated from ascites collected from passage growing on A/J mice. The cells were cultured using RPMI 1640, 10% FBS, 100 U/mL penicillin and 100 U/mL streptomycin.

Flow cytometry experiment

Cells were harvested from the culture. The cells suspended in FACS buffer (5% FBS, 0.1% NaN_3 in PBS) were incubated with 1:20 diluted mice sera on ice for 30 min. The cell suspension was centrifuged at 1600 rpm, 5 min at 4°C to remove the unbound antibodies. The cell-bound IgG antibodies were then labeled with goat anti-mouse IgG conjugated with FITC (BioLegend, 405305) for 30 min. The excess secondary antibody was washed out and the cells were suspended in FACS buffer. Acquisition of cells was performed with LSR II (BD), and data was analyzed with FlowJo® software (Tree Star Inc.).

SDS-PAGE gel analysis

The viral capsid samples (100 µg of each capsid protein) were loaded into 0.7% agarose gel in PBS with SYBR Safe DNA gel stain as a staining reagent for the encapsulated RNA. The electrophoresis was performed in TEA buffer at 4°C for 4 hours. After visualizing the encapsulated RNA bands by UV light, the gel was stained with Coomassie blue stain to detect the capsid protein.

Conjugation of GD2 derivative 3 with mQβ

A solution of mQβ (5 mg, 0.35 μmol subunit, 1.40 μmol reactive amine) in 0.1 M K-Phos buffer pH 8.0 (0.5 mL) was cooled on an ice batch, followed by the addition of GD2 **3** (100 μL from a 100 mg mL^{-1} stock solution in H_2O). The mixture was allowed to warm to room temperature and was gently inverted several times to ensure that the reactants were mixed. The reaction was incubated at 37 ℃overnight. The unreactive antigens were separated from conjugates by centrifugal filtration against 0.1 M K-Phos buffer (pH 7.0, 0.1 M). The extent of particle modification was determined by LC-MS and electrophoretic analysis. The total protein concentration was determined by Bradford assay against BSA standards. Percent protein recovery was 65–75 %.

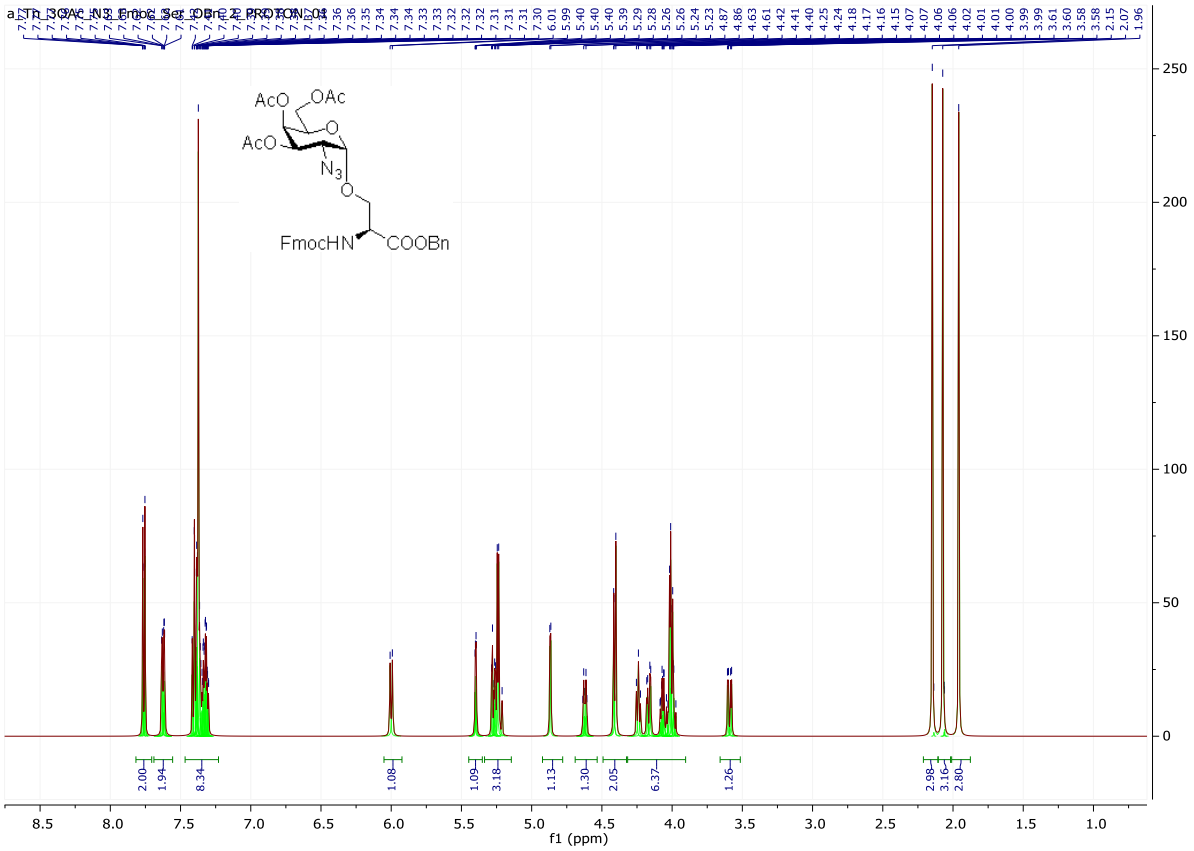
Conjugation of RBD peptides with mQβ

Acrylic ketone linker **6** (0.39 mg, 1.8 μmol, 1.4 eq. to Qβ reactive amine) was dissolved in DMSO and added to a solution of 3.7 mg mQβ (5.12 mg/ml, 0.0014 μmol protein, 1.3 μmol reactive amine) in KPB buffer, pH=8 at 10% V/V final DMSO concentration. The reaction was incubated at r.t with continuous shaking for 1h. The reaction mixture was worked up by ultracentrifugation against KPB buffer pH=8 for 5 times. The peptide **4** was dissolved in DMSO (6.76 mg, 2 eq. per reactive amine) and added to the solution of mQβ-linker in KPB (10% V/V final DMSO concentration) and the reaction was incubated at 37°C overnight. After that, the reaction mixture was worked up with ultracentrifugation against PBS buffer pH=7 for 5 times. The protein concentration was quantified with the Bradford assay and the peptide loading was calculated based on the LC-MS result.

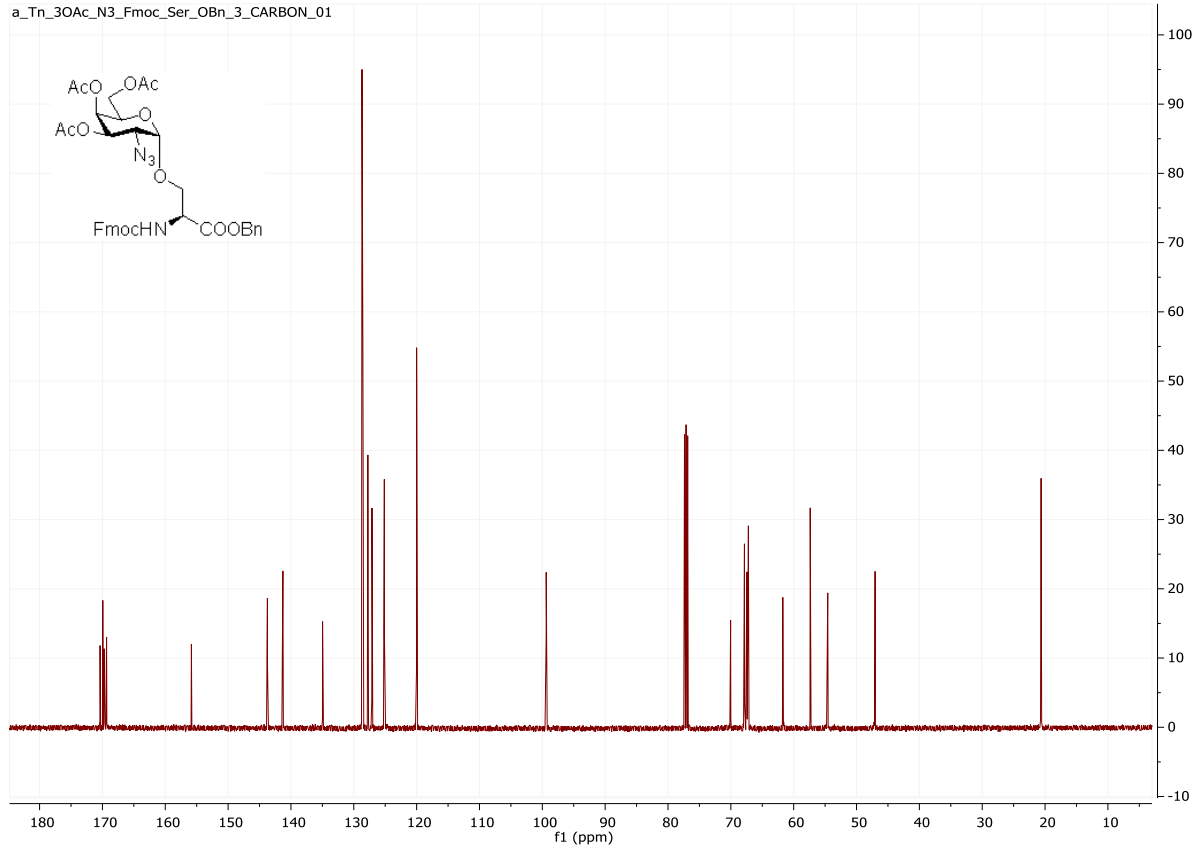
For conjugation of RBD peptide **5**, to a solution of peptide **5** (10 mg, 4.74 μmol) in dry DMF (500 μL), DIPEA (1.7 μL, 9.5 μmol, 2 eq.) was added, followed by the addition of bis(4-nitrophenyl) ester linker **7** (5.5 mg, 14.2 μmol, 3 eq.). The mixture was stirred for 2 h as indicated by MS analysis of complete consumption of **5**. Then the reaction was concentrated, and the desired peptide was precipitated in CH_2Cl_2. The peptide was washed with CH_2Cl_2 X 3 times (2 mL) and EtOAc X 3 times (2 mL) to remove the unreacted linker. The products were characterized by ESI HRMS. HRMS (ESI-MS): $C_{104}H_{136}N_{26}O_{34}S_2$ $[M+2H]^{+2}$ calcd: 1179.9667, obsd: 1179.9667, $[M+NH_4+H]^{+2}$ calcd: 1187.9783, obsd: 1187.9764. A solution of mQβ (5 mg, 0.35 μmol subunit, 1.40 μmol reactive amine) in 0.1 M K-Phos buffer pH 8.0 (0.5 mL) was cooled on an ice batch, followed by the addition of the linker functionalized peptide **5**. The mixture was allowed to warm to room temperature and was gently inverted several times to ensure that the reactants were mixed. The reaction was incubated at 37 ℃ overnight. The unreactive antigens were separated from conjugates by centrifugal filtration against 0.1 M K-Phos buffer (pH 7.0, 0.1 M). The extent of particle modification was determined by LC-MS and electrophoretic analysis.

NMR spectra

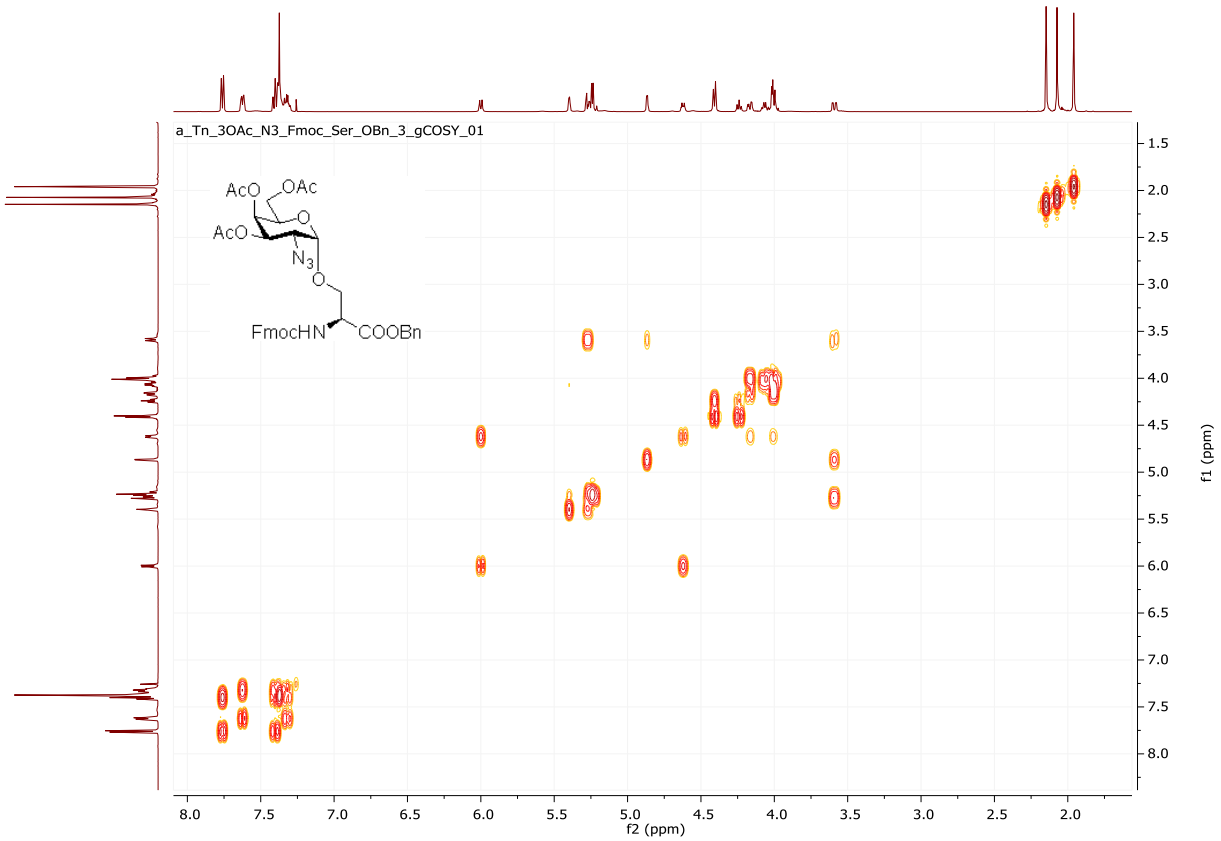
*N-(Fluoren-9-ylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl-L-serine benzyl ester (**SI-3**):*



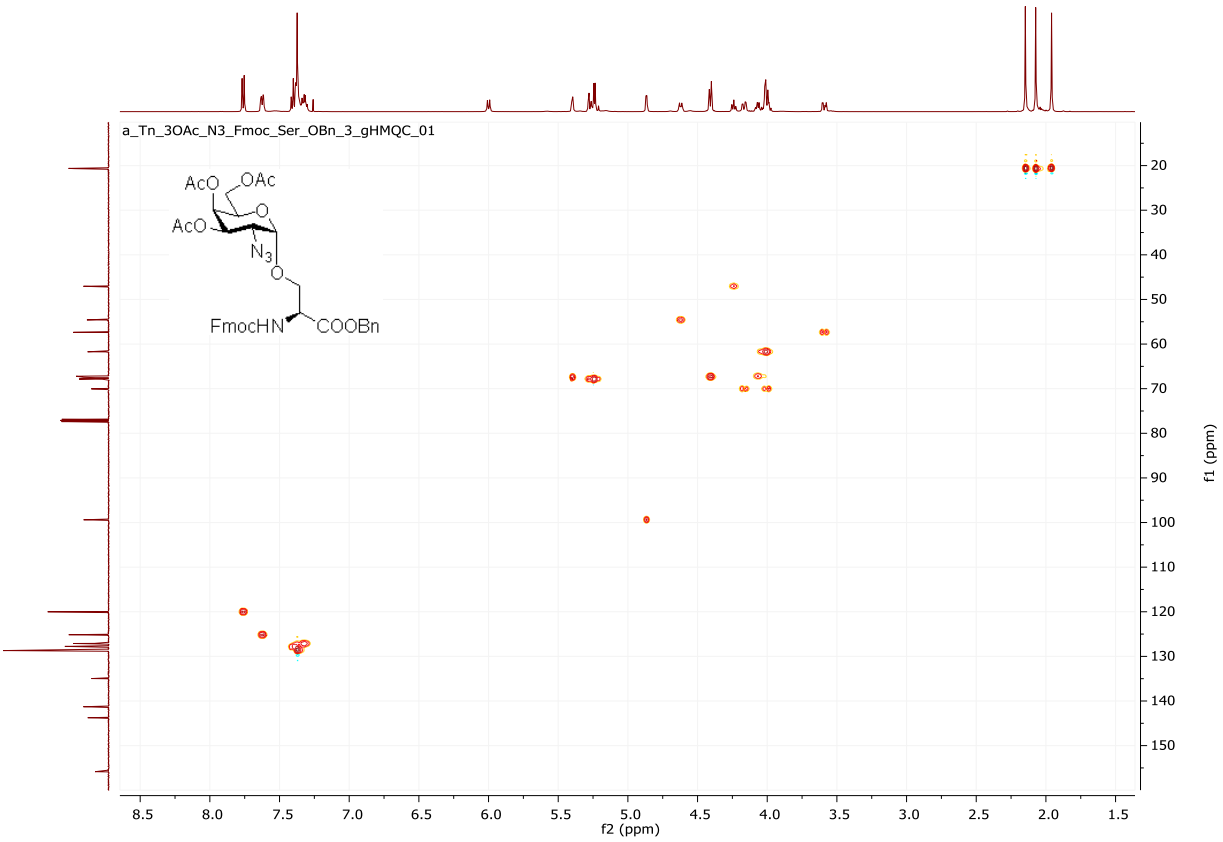
^{1}H NMR spectrum of compound SI-3



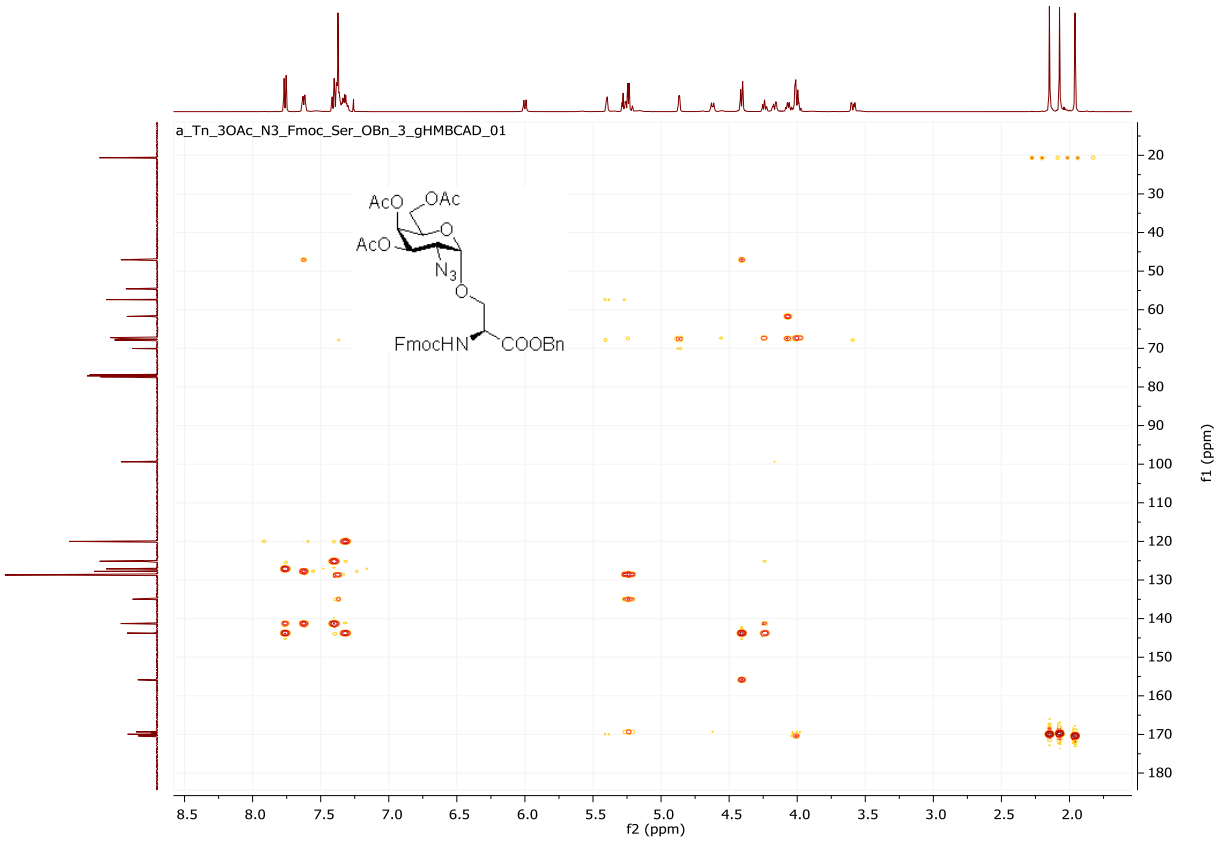
^{13}C NMR spectrum of compound SI-3



^{1}H-^{1}H COSY NMR spectrum of compound SI-3

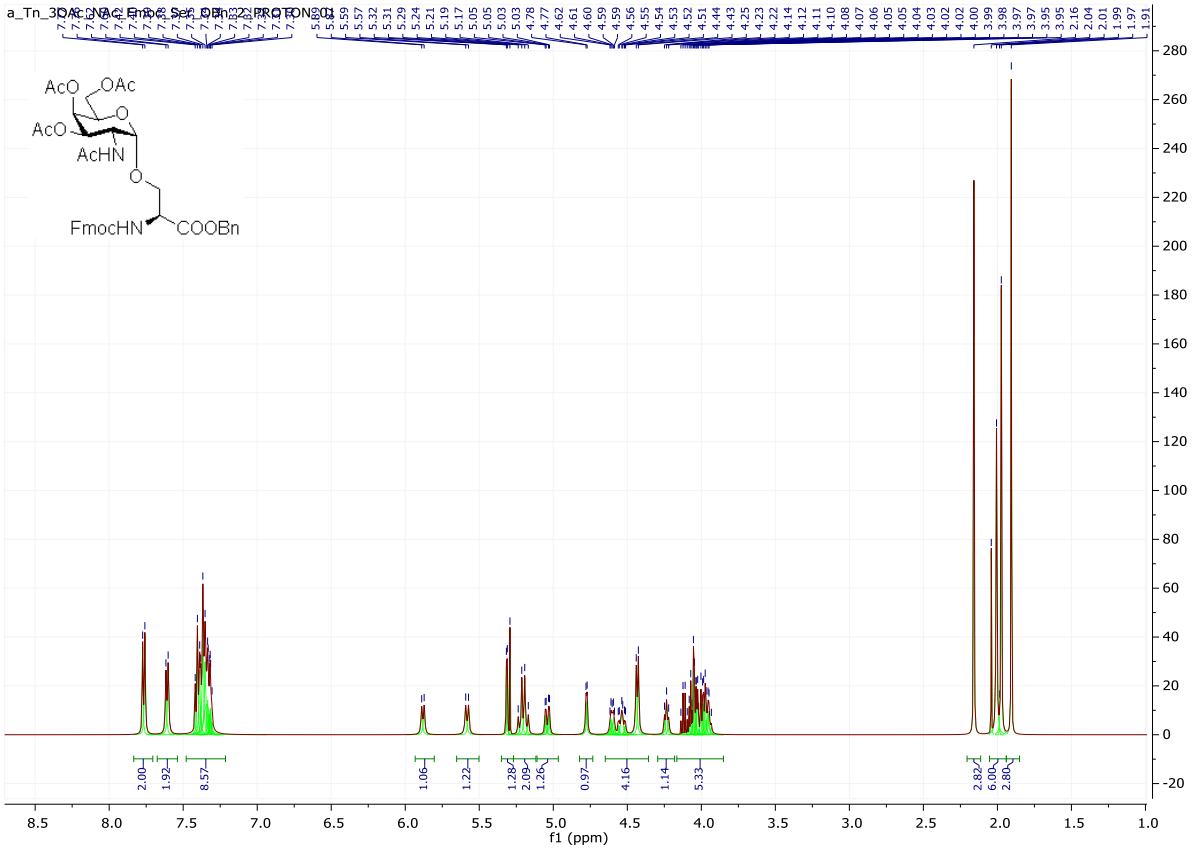


gHMQC NMR spectrum of compound SI-3

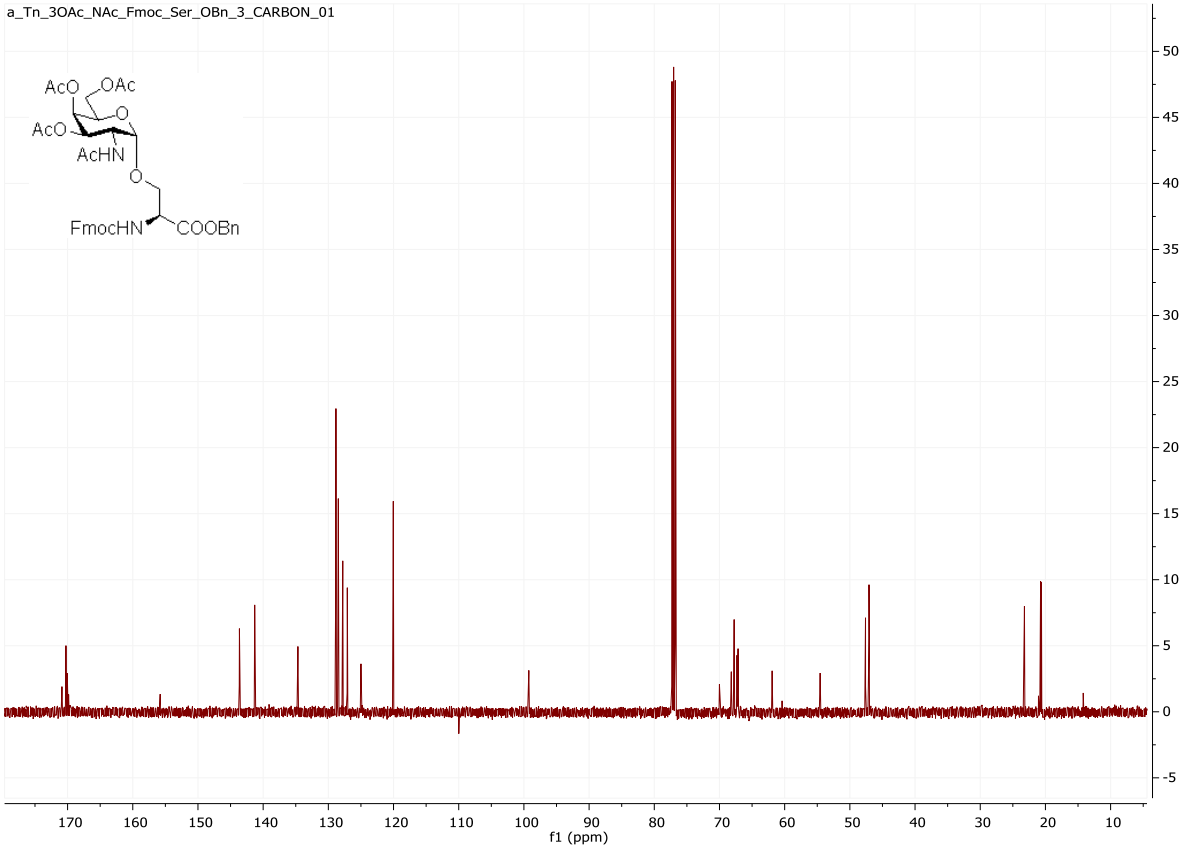


gHMBC NMR spectrum of compound SI-3

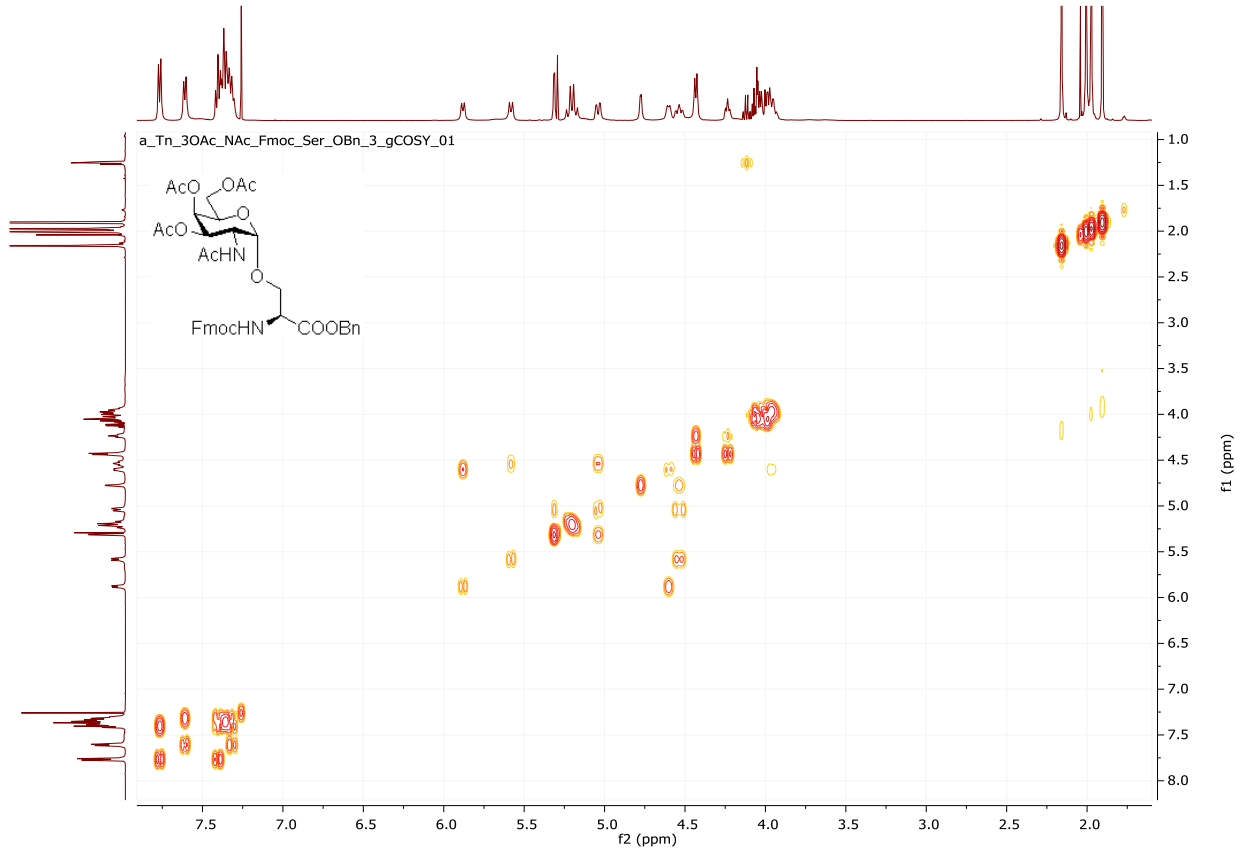
*N-(Fluoren-9-ylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-serine benzyl ester (**SI-4**):*



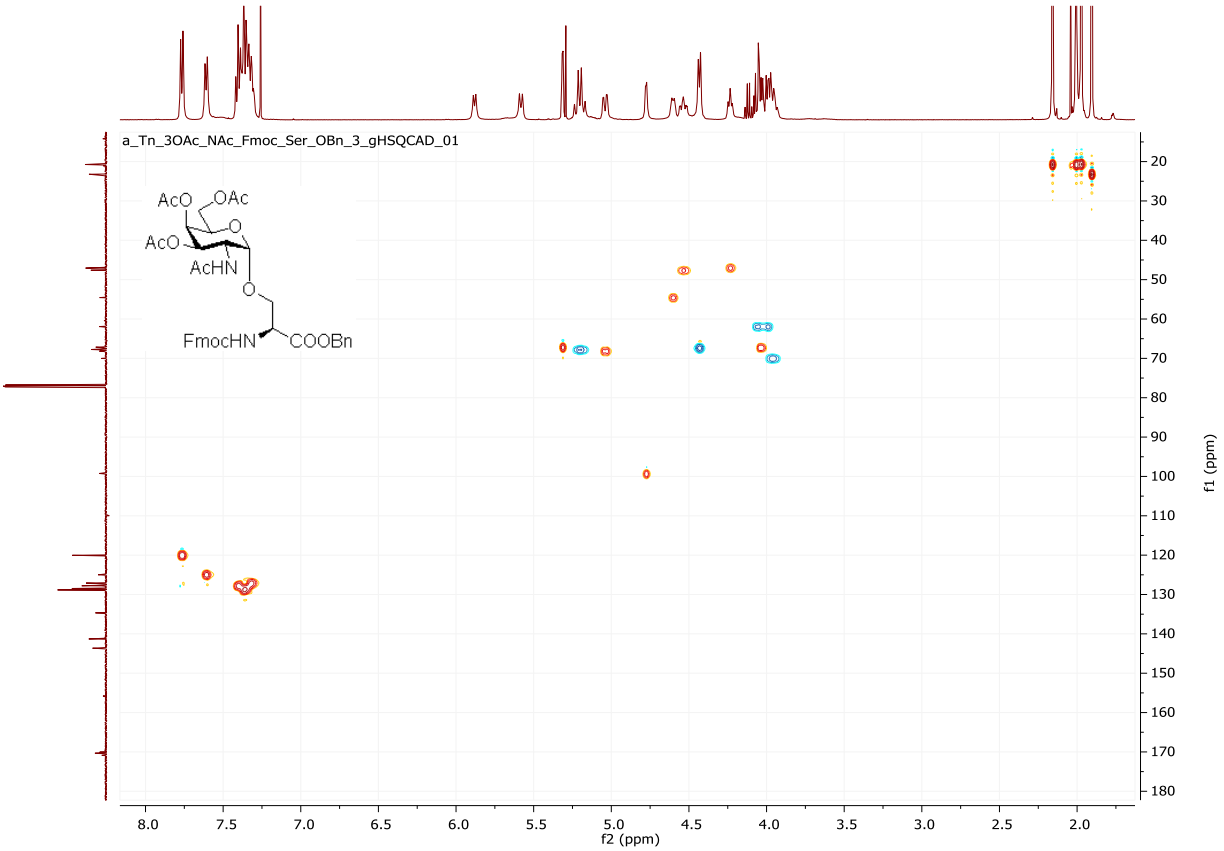
^{1}H NMR spectrum of compound SI-4



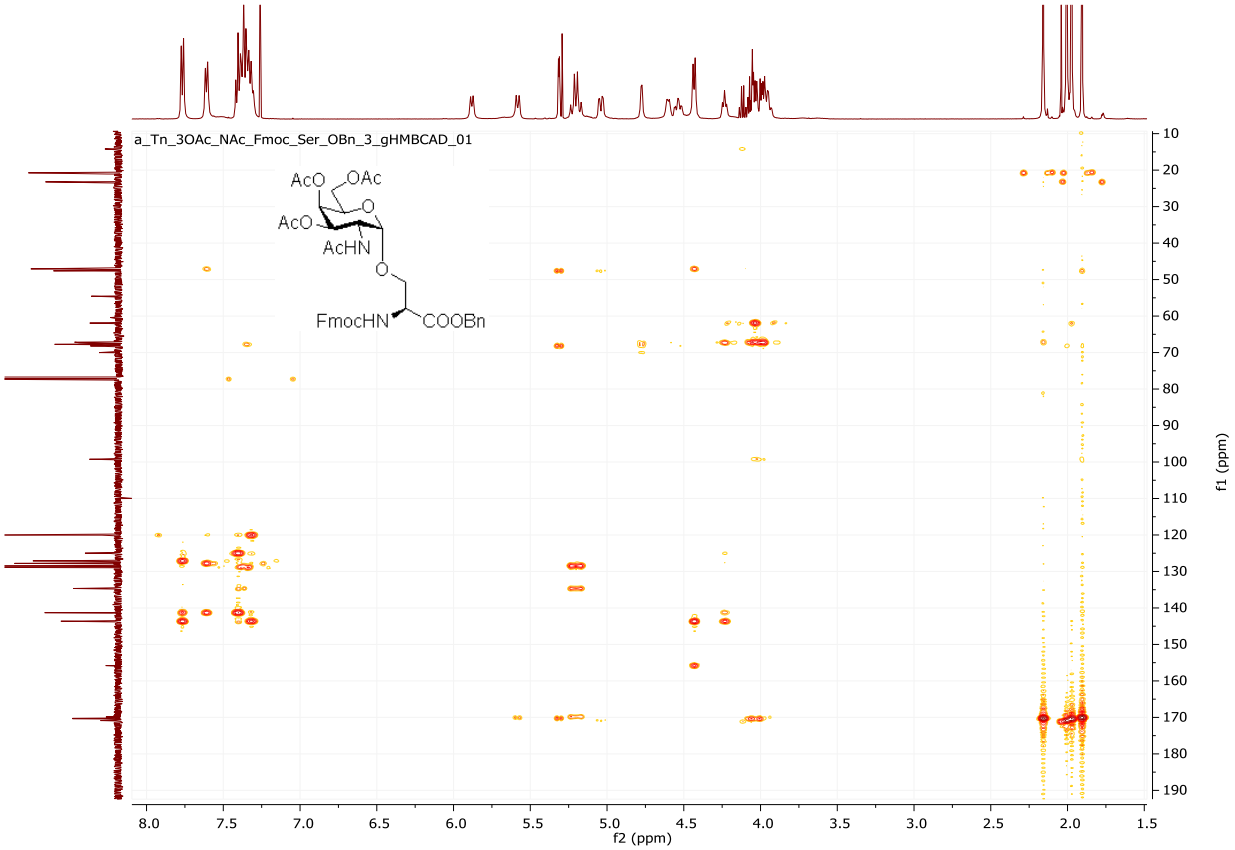
^{13}C NMR spectrum of compound SI-4



^{1}H-^{1}H COSY NMR spectrum of compound SI-4

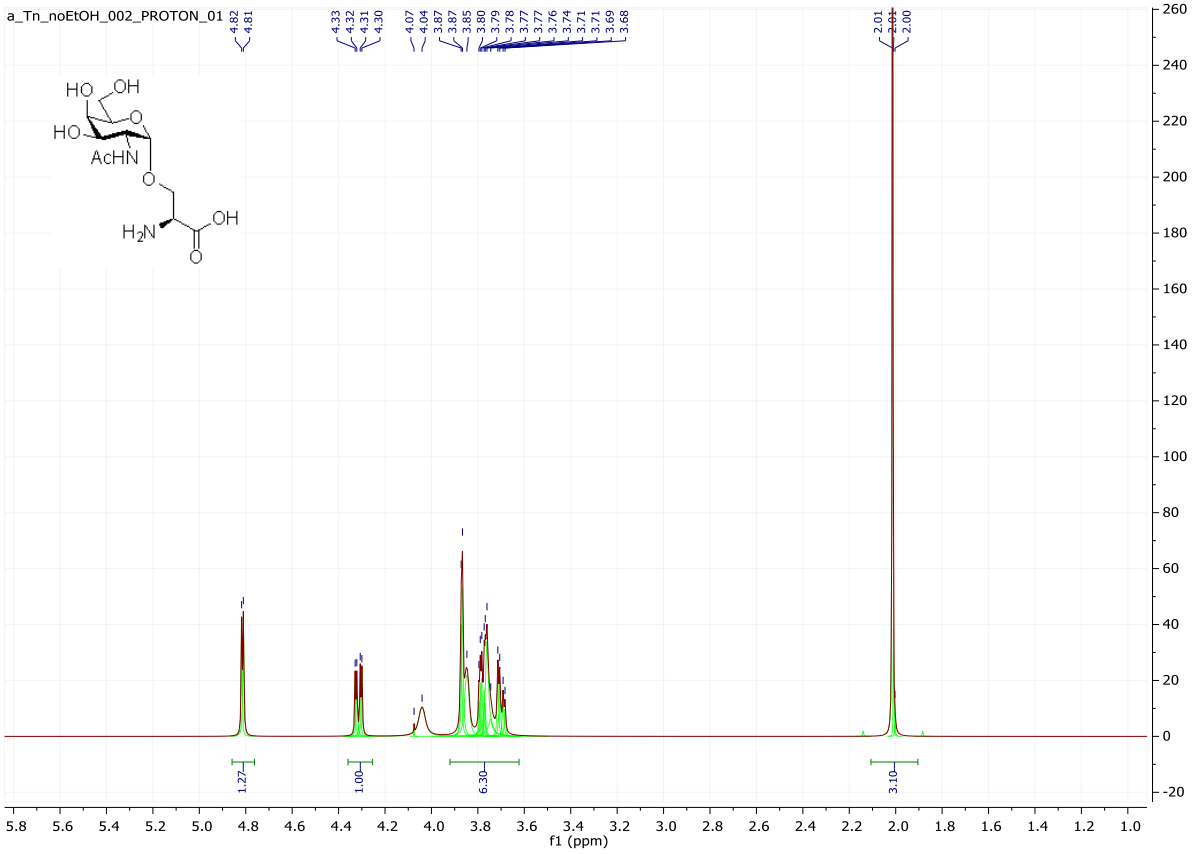


gHMQC NMR spectrum of compound SI-4

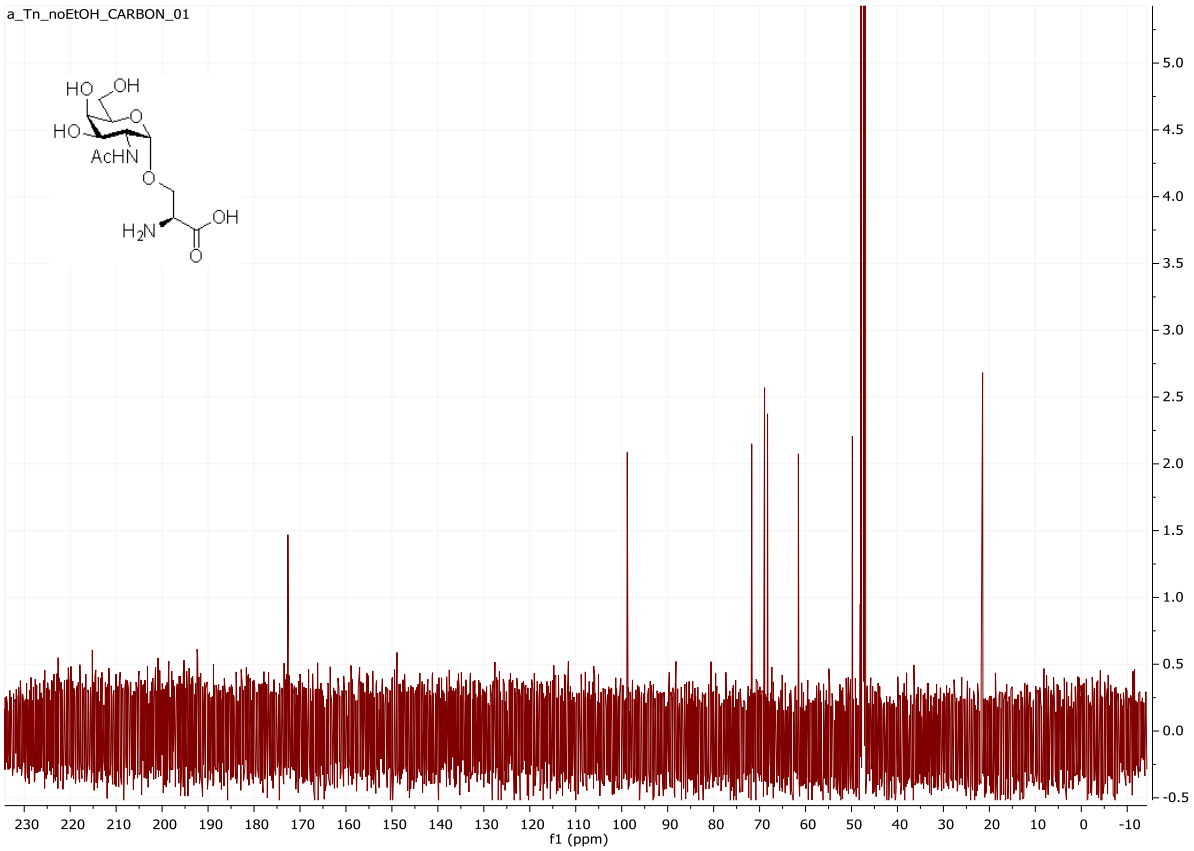


gHMBC NMR spectrum of compound SI-4

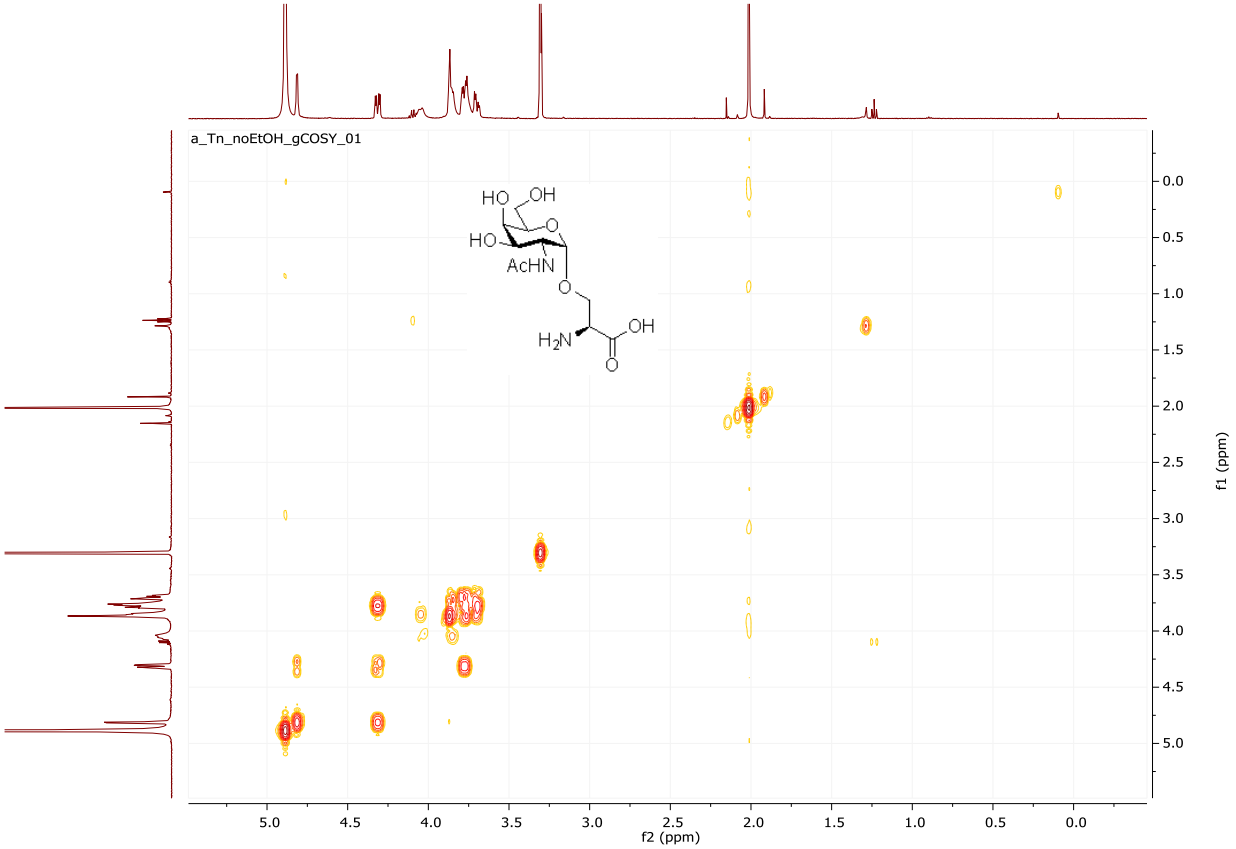
*O-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-serine (**SI-5**):*



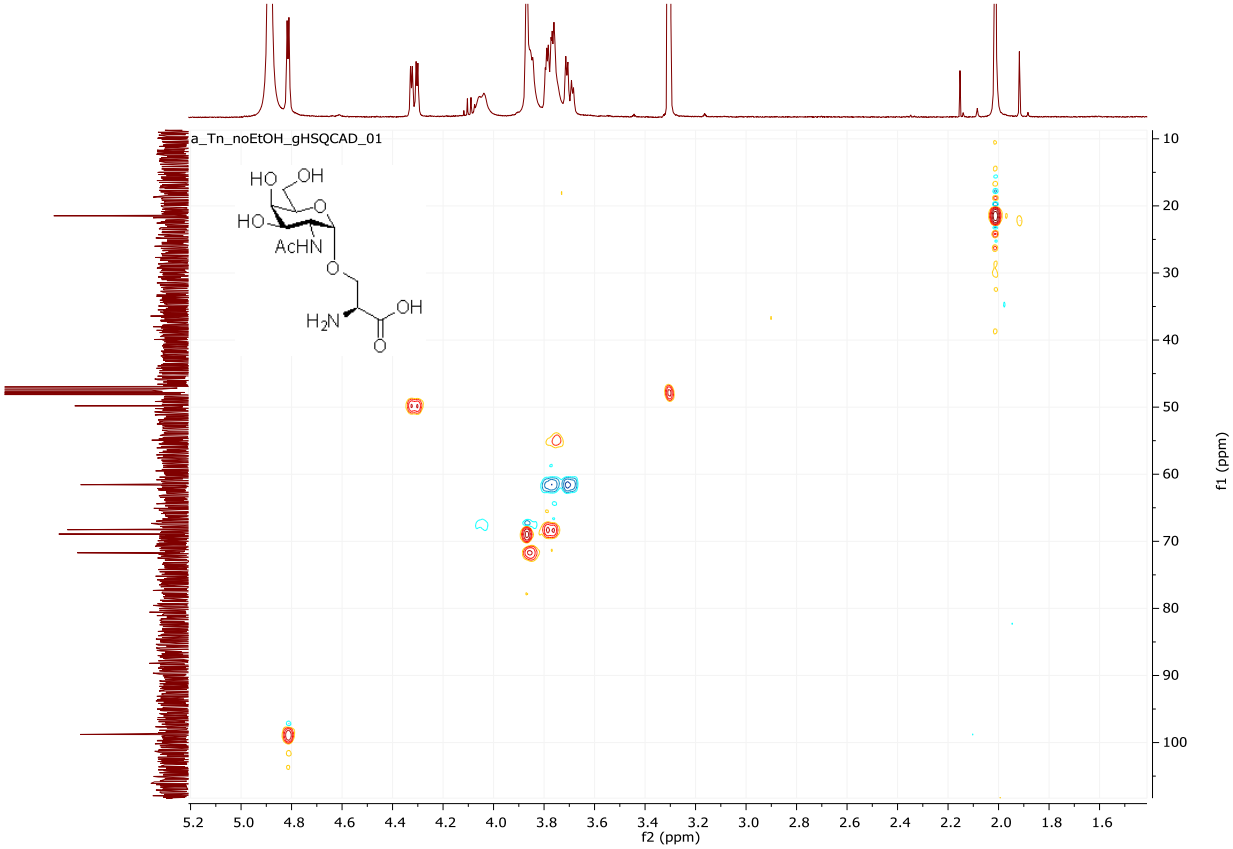
^{1}H NMR spectrum of compound SI-5



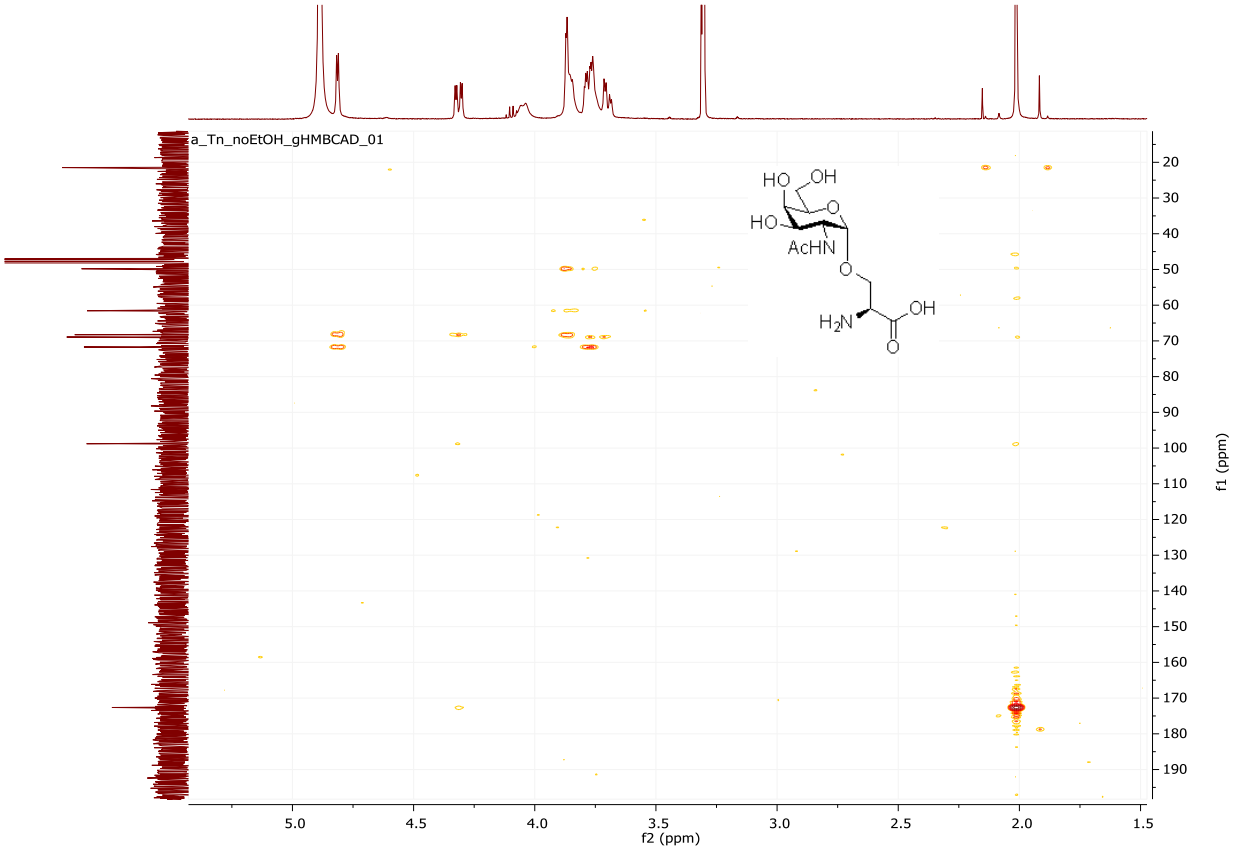
^{13}C NMR spectrum of compound SI-5



^{1}H-^{1}H COSY NMR spectrum of compound SI-5

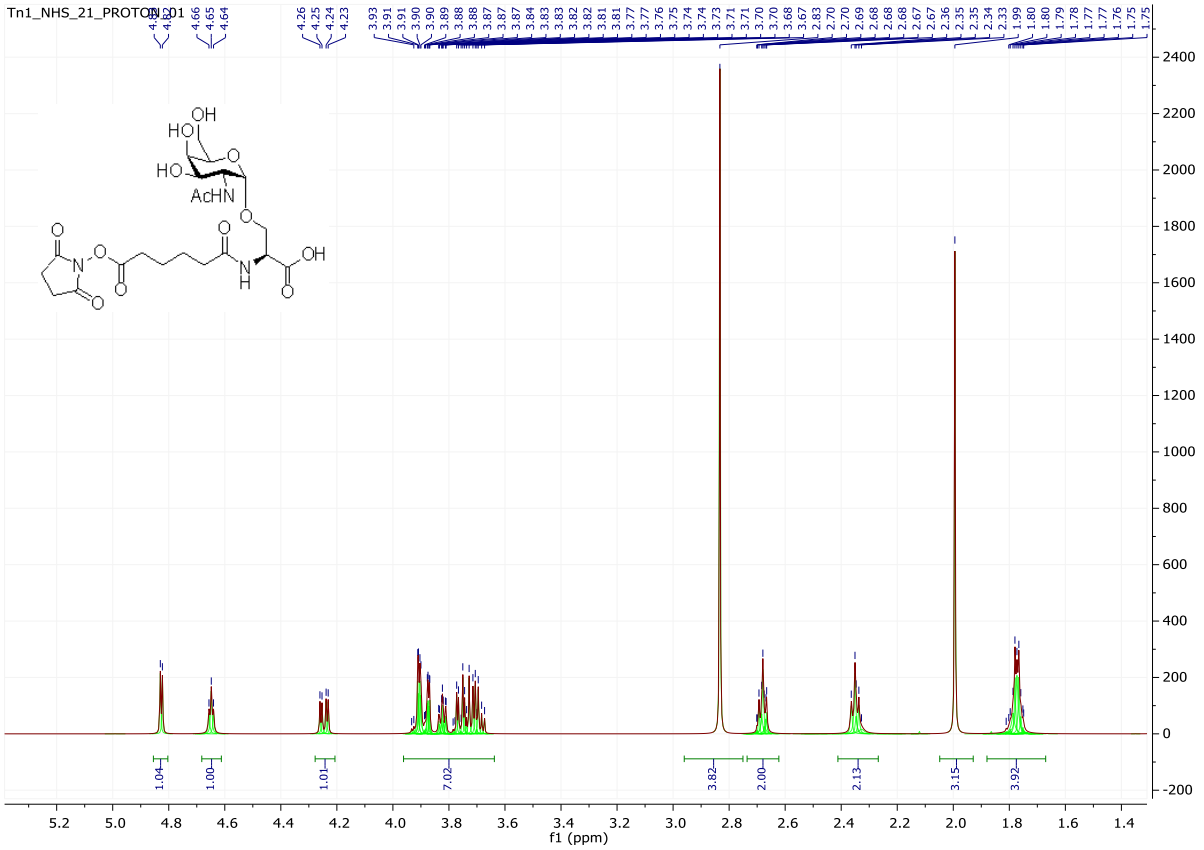


gHMQC NMR spectrum of compound SI-5

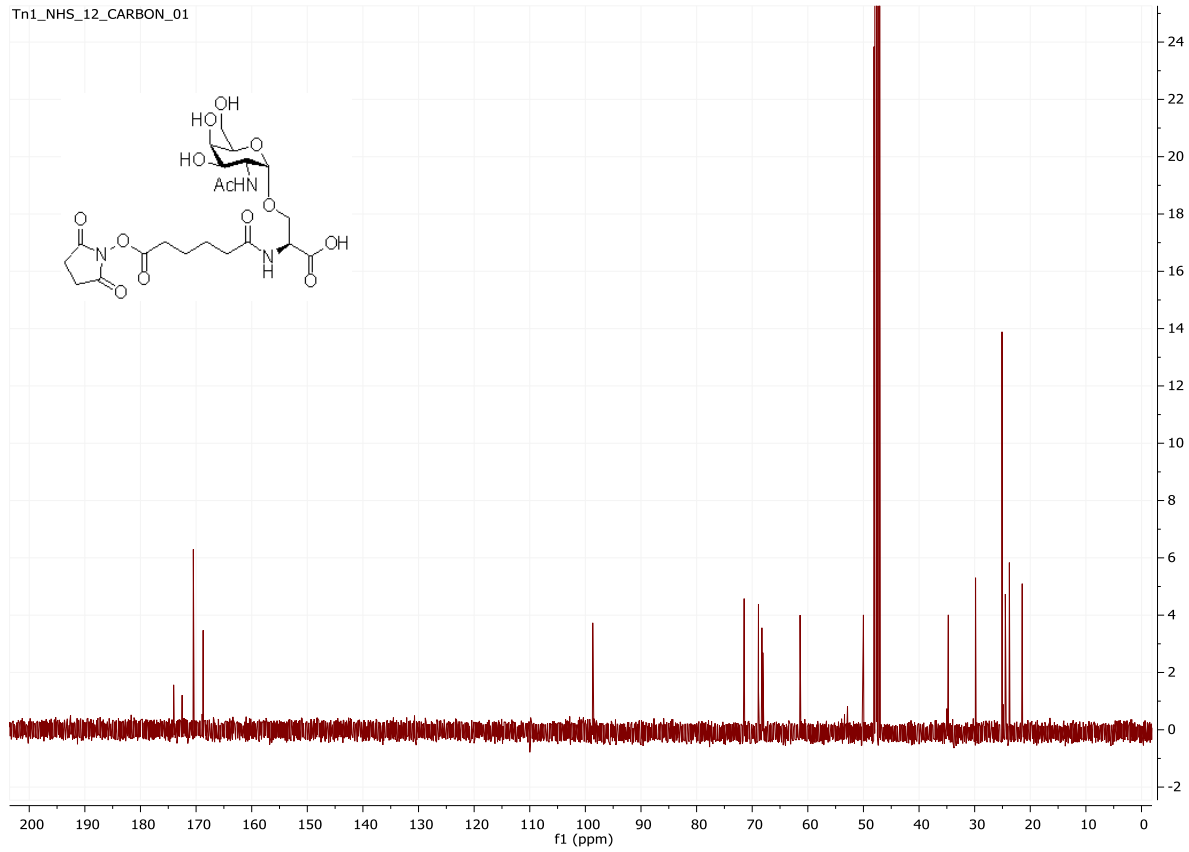


gHMBC NMR spectrum of compound SI-5

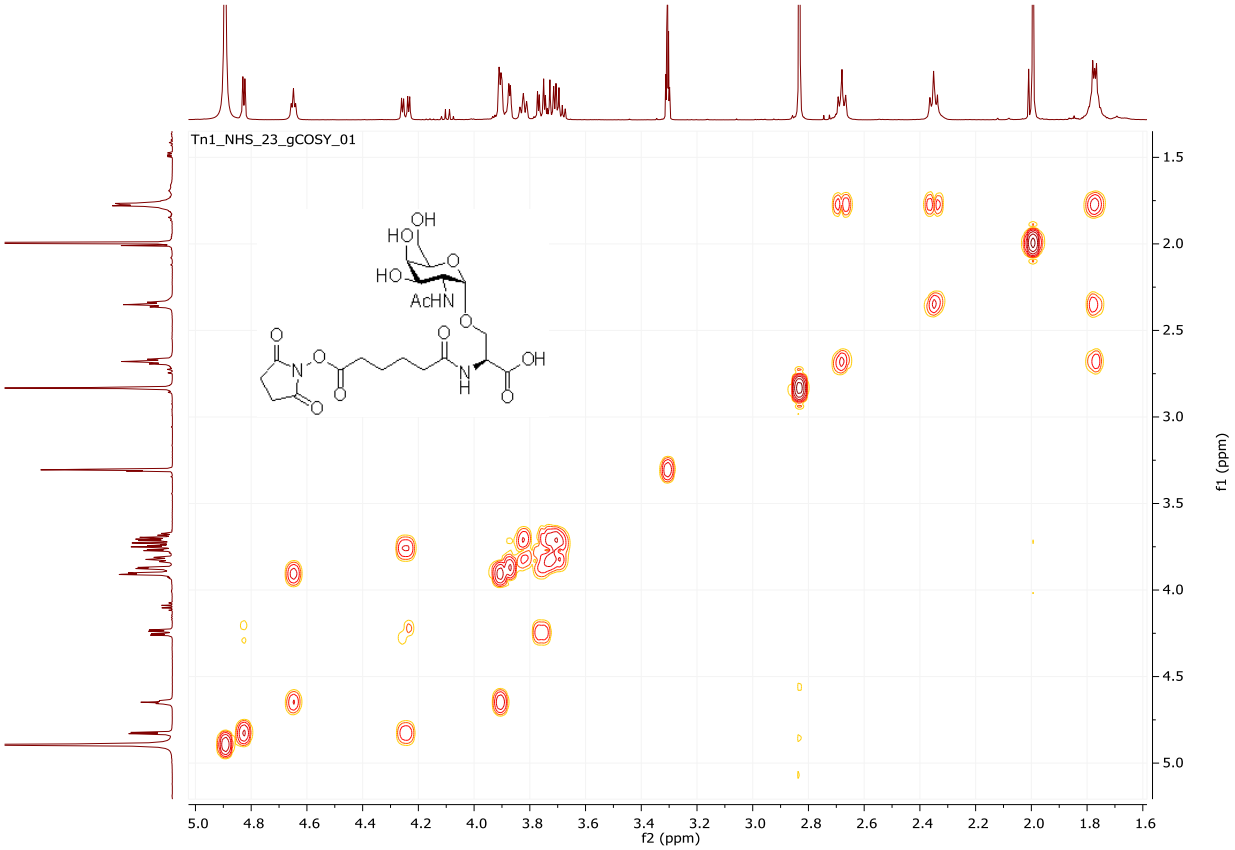
*N-(N-Hydroxysuccinimidyl-adipoyl)-O-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-serine (NHS Tn **1**):*



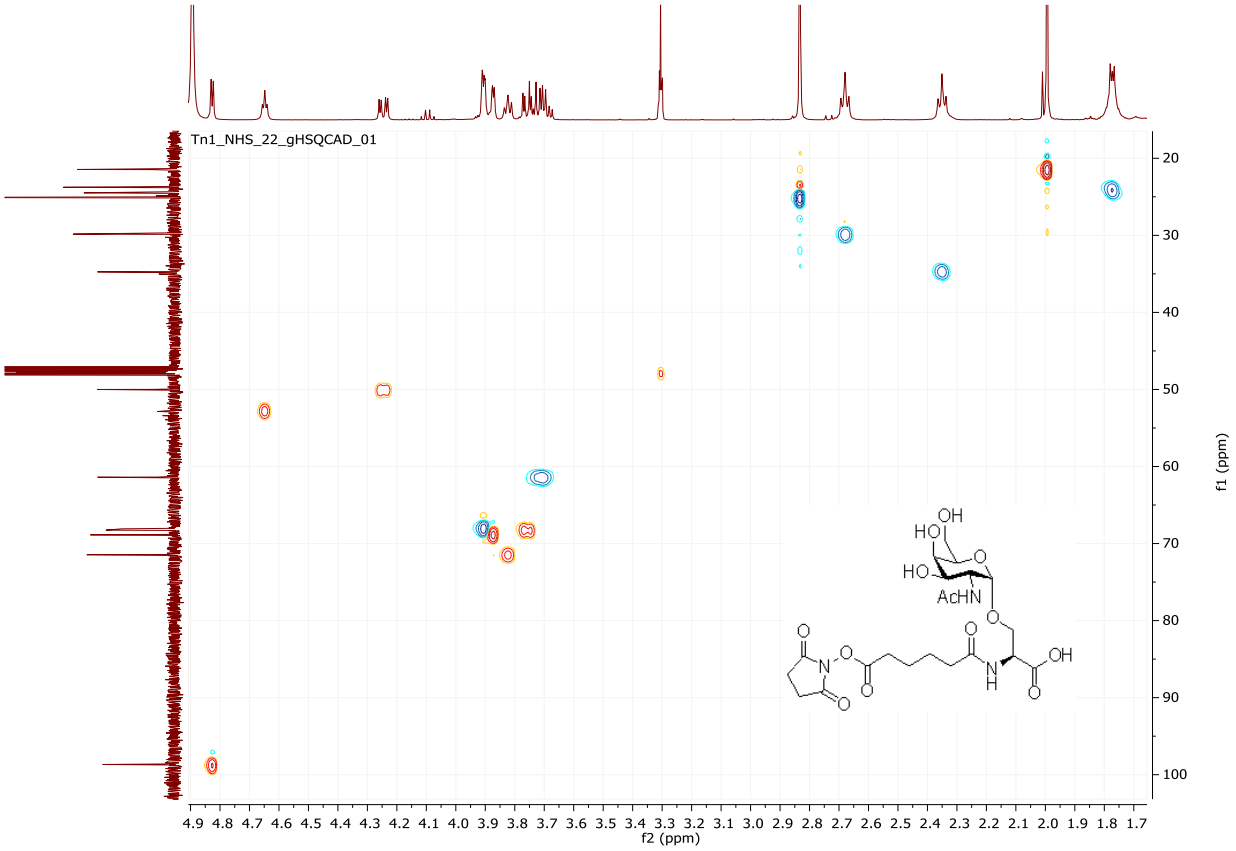
^{1}H NMR spectrum of compound NHS Tn **1**



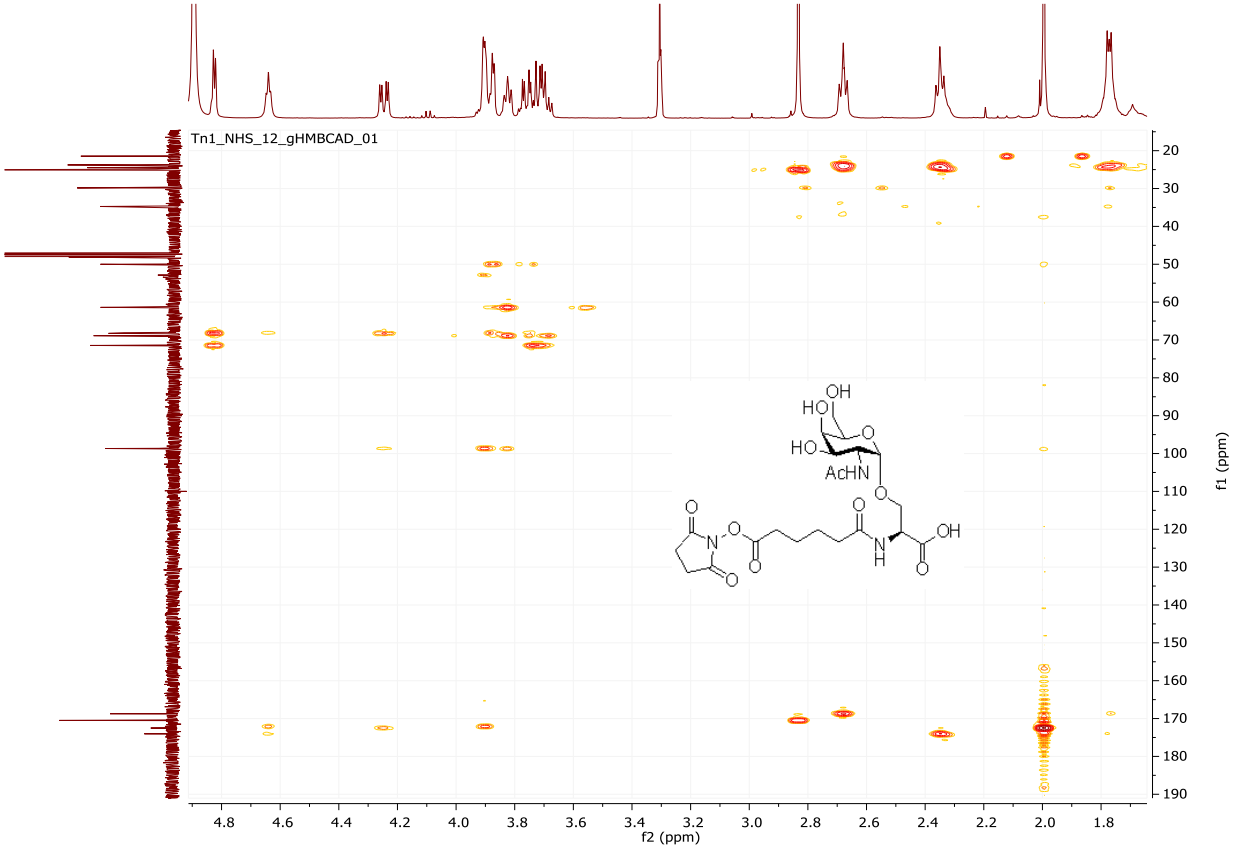
^{13}C NMR spectrum of compound NHS Tn **1**



^{1}H-^{1}H COSY NMR spectrum of compound NHS Tn **1**



gHMQC NMR spectrum of compound NHS Tn **1**



gHMBC NMR spectrum of compound NHS Tn **1**

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