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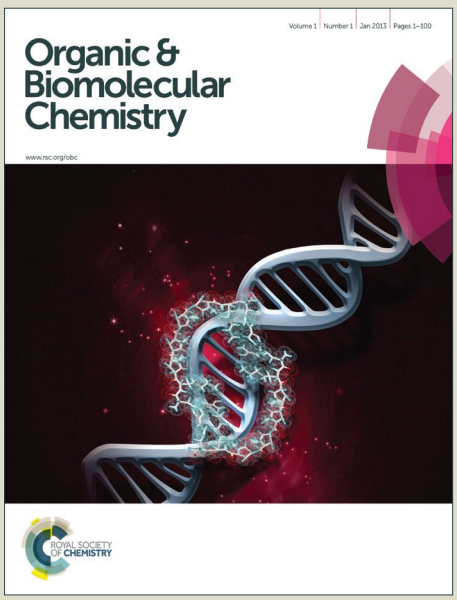

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Synthetic and Immunological Studies of *N*-Acyl Modified *S*-Linked STn Derivatives as Anticancer Vaccine Candidates†

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It is well known that tumor cells express some aberrant glycans, termed tumor-associated carbohydrate antigens (TACAs). TACAs are good targets for the development of carbohydrate-based anticancer vaccines. However, one of the major problems is that carbohydrate antigens possess weak immunogenicity. To tackle this problem, a number of unnatural *N*-modified *S*-linked STn analogues were designed and prepared. Reaction of the modified STn disaccharides with bifunctional adipic acid *p*-nitrophenyl diester provided the corresponding activated esters, which was followed by the conjugation with keyhole limpet hemocyanin (KLH), affording the corresponding protein conjugates. The immunological properties of these glycoconjugates were evaluated in mouse model. The results showed that the modified glycoconjugates stimulated the production of IgG antibodies capable of recognizing the naturally occurring STn antigen, benefiting the discovery of carbohydrate-based anticancer vaccine candidates.

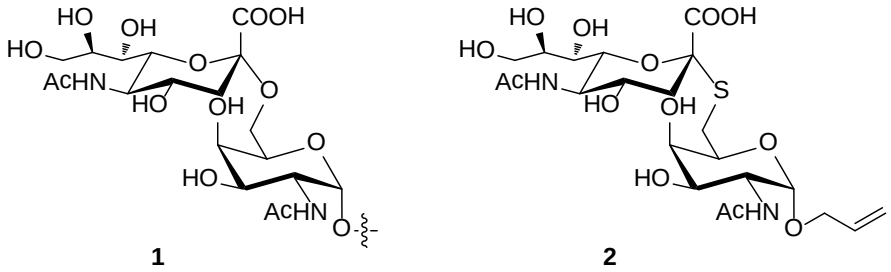
Introduction

Tumor-associated carbohydrate antigens (TACAs), which are overexpressed on the the surface of tumor cells, are correlated with tumor cell adhesion and metastasis,[1, 2] and are regarded as important targets for anticancer vaccine development.[3-11] However, as self-antigens, natural TACAs cannot be recognized and processed effectively by the immune system, so they exhibit poor immunogenicity.[12] In 1986, Jennings' group reported that the modification of carbohydrate antigen structures improved the immunogenicity of the vaccine against group B meningococcal meningitis.[13, 14] Later, they applied this strategy to polysialic acid with longer chain, a TACA which is expressed abundantly on the small cell lung cancer cells. Vaccination with modified polysialic acid-KLH (keyhole limpet hemocyanin) conjugate resulted in a consistent high-titer antibody response and the induced IgM antibodies cross-reacted with natural polysialic acid while vaccination with natural polysialic acid-KLH conjugate rarely produced antibodies.[15] Recently, more TACAs were modified in different ways, proving the validity of this protocol.[16-28]

STn antigen **1** (Figure 1), an *O*-linked disaccharide [NeuAcα-(2-6)GalNAc], is expressed on a wide range of tumor cells such as breast, prostate, and ovarian carcinomas but is rarely observed in normal tissues. Expression of STn is usually associated with poor prognosis[29-32] and metastatic progression.[33] Therefore, STn-based vaccines are promising vaccine candidates. Although the natural STn-KLH conjugate Theratope® failed to increase the median time of survival of breast cancer patients in the phase III trial,[34] considerable efforts have been made to improve the efficacy. In 2010, three *N*-modified STn-KLH conjugates were synthesized by Lin's group. The immunoassay results revealed that the modified STn-KLH conjugates were more immunogenic than natural STn-KLH conjugate, and the antisera could cross-react with natural STn. Among them, the *N*-propionyl STn-KLH conjugate was demonstrated to be the most immunogenic.[35] At the same time, a small library containing forty structurally modified STn antigens was constructed by us. The immunoassay results on mice showed that three fluorine-substituted STn derivatives significantly improved the immunogenicity and their antisera reacted strongly with

the LS-C human colon cancer cells expressing natural STn.[36]

Sulfur-linked oligosaccharides are excellent mimics of oxygen-linked oligosaccharides. The sulfur atom may act as a hydrogen bond acceptor which could play an important role in the binding of the ligand as in the natural oligosaccharides. As the *S*-linkage is highly flexible between glycosyl units, *S*-linked oligosaccharides possess more low-energy conformers in solution than the natural oligosaccharides.[37] Furthermore, substitution of the glycosidic oxygen atom by sulfur atom enhances the stability of the glycosidic linkage since the *S*-linkage is resistant to endogenous glycosidases. *S*-Linked oligosaccharides have been demonstrated to inhibit the activity of glycosidases in several cases.[38-40] Therefore, as non-self antigens, *S*-linked oligosaccharide antigens may be more immunogenic and stimulate the production of antibodies capable of recognizing natural antigens. Some researchers have reported the synthesis and immunological evaluation of vaccines based on *S*-linked oligosaccharide antigens.[41-46] In 2009, a *S*-linked STn antigen **2** (Figure 1) was synthesized by us.[47] Herein we want to design and synthesize several *S*-linked STn antigens with further *N*-acyl modifications, and evaluate the immunological properties of the resultant KLH conjugates.



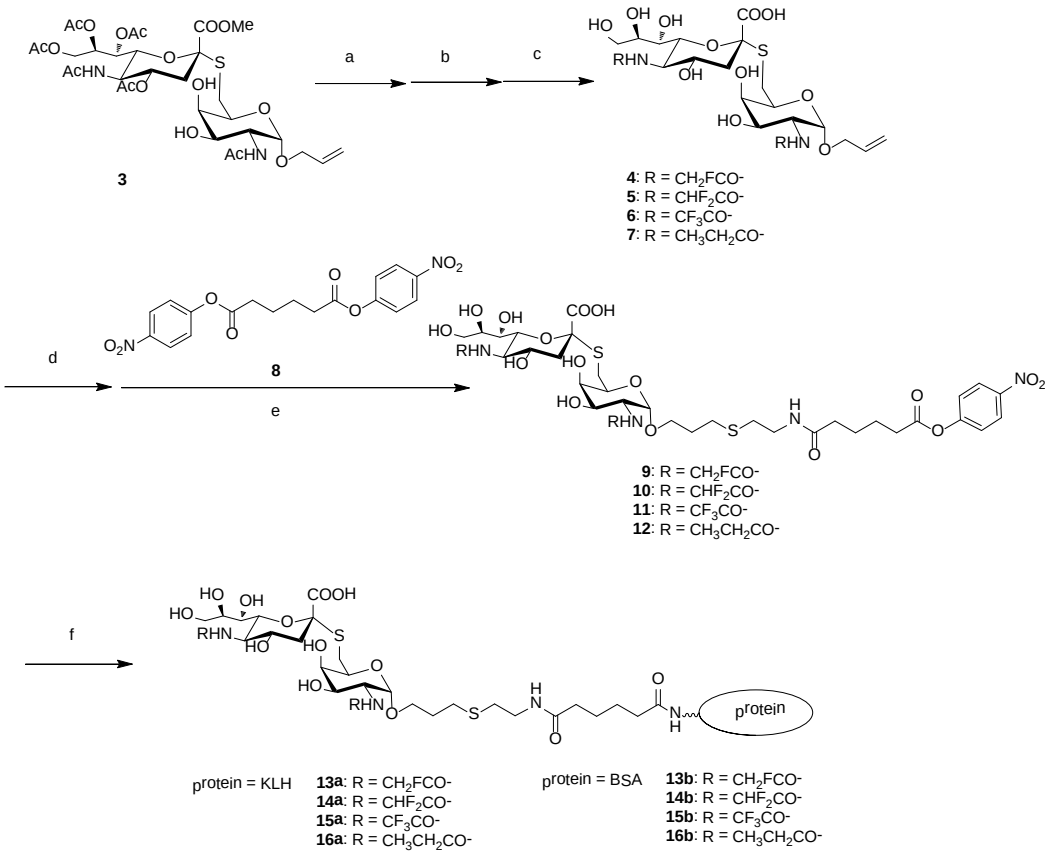
1 **2**

Figure 1. Structures of natural STn antigen and *S*-linked STn antigen **2**

Results and Discussion

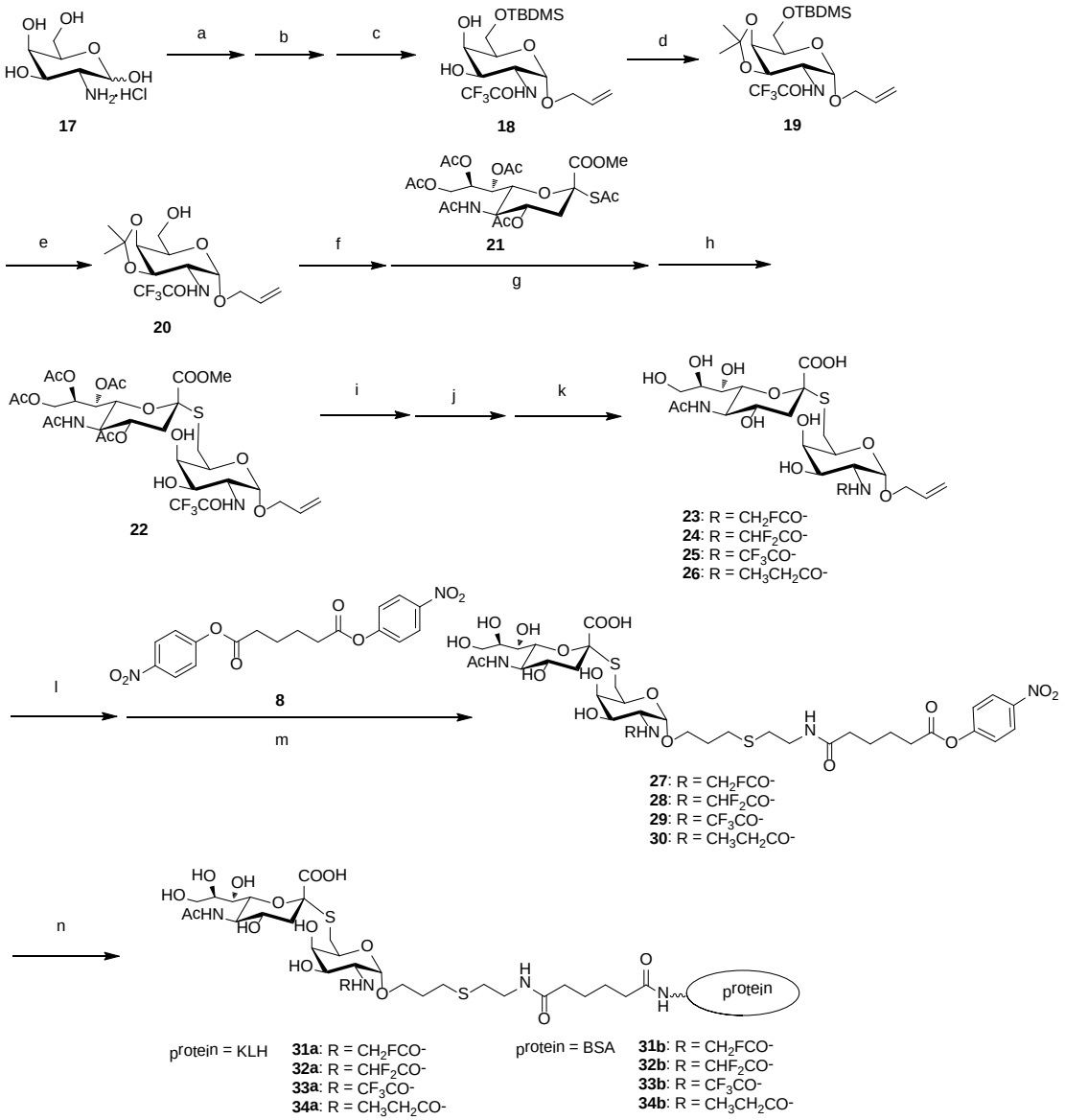
Based on the structure of *S*-linked STn antigen **2**, further modifications on *N*-acyl substitutions were designed. Since the *N*-propionyl and fluorinated modifications could enhance the immunogenicity,[35, 36] propionyl, fluoroacetyl, difluoroacetyl, and trifluoroacetyl substituents were introduced to the target molecules. For this purpose,

disaccharide **3**[47] was chosen as the starting material for the preparation of the *S*-linked STn analogues with *N*-modifications on both galactosamine moiety and sialic acid moiety (Scheme 1). The *O*-acetyl and *N*-acetyl groups in **3** were removed by exposure to sodium methoxide in methanol at room temperature and 2 M sodium hydroxide solution under reflux sequentially. The subsequent coupling reactions with methyl fluoroacetate, methyl difluoroacetate, methyl trifluoroacetate, and propionic anhydride produced the *N*-modified *S*-linked STn derivatives **4**-**7**, respectively. To avoid the oxidation of sulfur atom by ozone, a linkage that is different from our previous work[36] was needed to couple the carbohydrate antigens to carrier proteins. Thus, photoaddition[48-55] of 2-aminoethanethiol to the allyl glycosides **4**-**7** yielded the amine-functionalized glycosides, which were treated with homobifunctional linker **8**,[56-59] affording the corresponding esters **9**-**12**. Compounds **9**-**12** were then coupled to carrier proteins by incubation in PBS buffer. In this way, the KLH conjugates **13a**-**16a** and the bovine serum albumin (BSA) conjugates **13b**-**16b** were obtained after dialysis against PBS buffer.



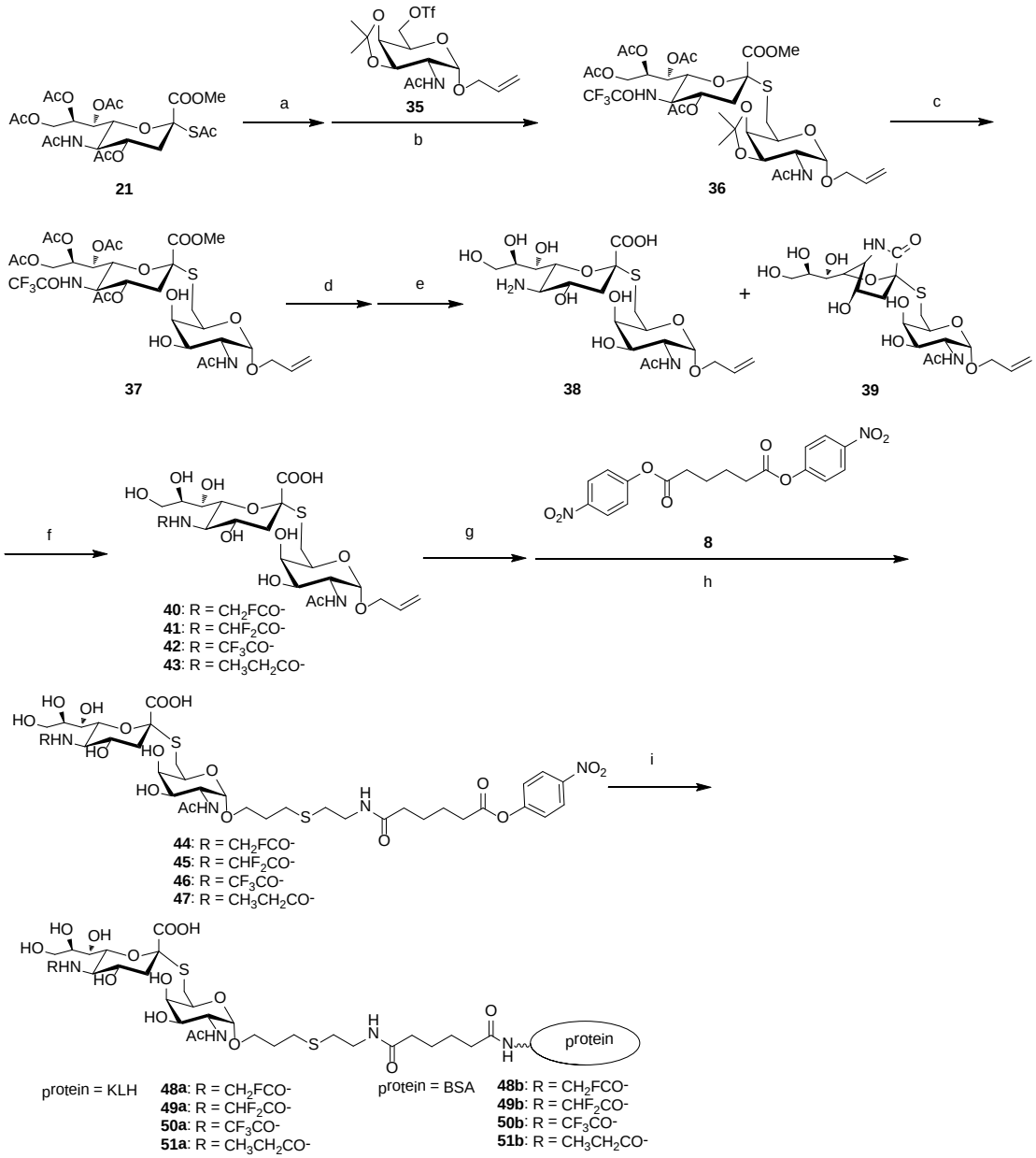
Scheme 1. The synthesis of glycoconjugates **13a**-**16a** and **13b**-**16b**. Reagents and conditions: a) NaOMe (30%), MeOH, rt; b) 2 M NaOH aqueous solution, reflux; c) methyl ester of corresponding carboxylic acid, MeOH, TEA, Ar, reflux or propionic anhydride, $NaHCO_3$, MeOH, 0 ^oC, 93% for **4**, 95% for **5**, 74% for **6** and 94% for **7**, over three steps; d) $HSCH_2CH_2NH_2 \cdot HCl$, MeOH, UV (254 nm), rt; e) **8**, DMF, TEA, Ar, rt, 35% for **9**, 27% for **10**, 70% for **11** and 60% for **12**, over two steps; f) PBS buffer, KLH or BSA, rt.

To prepare the *S*-linked antigen derivatives with *N*-modifications only in galactosamine moiety, the trifluoroacetyl group was utilized as the *N*-protective group in galactosamine building block (Scheme 2).[60] Galactosamine **17** was treated with methyl trifluoroacetate to give the trifluoroacetamide, without purification, which underwent the glycosylation reaction with anhydrous allyl alcohol using HCl as promoter.[61, 62] After complete conversion, the β-anomer and furanoside[63] were difficult to be separated from the α-anomer by column chromatography. Only after the primary hydroxyl functionality was masked with *t*-butyldimethylsilyl group, compound **18** as the desired α-configuration was readily isolated in 46% yield (over three steps). 3,4-*O*-Isopropylidenation of **18** afforded compound **19**.[64, 65] Removal of the *t*-butyldimethylsilyl group in **19** produced monosaccharide **20** with the 6-hydroxyl exposed. Subsequently, compound **20** was treated with triflic anhydride to form the 6-*O*-triflated intermediate, which was directly used for the next step reaction. The sialyl donor **21** was prepared following the reported procedure.[66] With both building blocks in hand, the coupling reaction between the triflate and the glycosyl donor **21** furnished the desired *S*-linked disaccharide, which was followed by de-isopropylidenation with 85% AcOH, affording compound **22**. Disaccharide **22** was sequentially treated with sodium methoxide and sodium hydroxide at room temperature to give the deprotected product. Eventually, the deprotected disaccharide was *N*-acylated and conjugated to carrier proteins to obtain the KLH conjugates **31a**-**34a** and the BSA conjugates **31b**-**34b** by the same protocol as described in the preparation of glycoconjugates **13a**-**16a**.



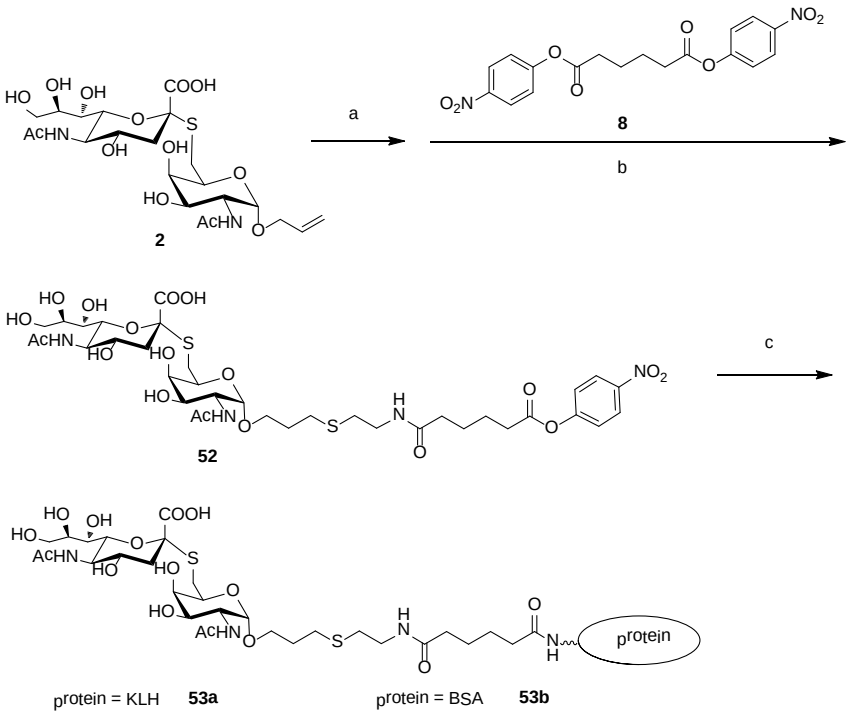
Scheme 2. The synthesis of glycoconjugates **31a**-**34a** and **31b**-**34b**. Reagents and conditions: a) CF_3COOMe, MeOH, TEA, rt; b) 8% HCl solution in allyl alcohol, 110 ^oC; c) TBDMSCl, Py, rt, 46% over three steps; d) DMP, CSA, rt, 51%; e) AcOH, $TBAF{\cdot}3H_2O$, THF, 50 ^oC, 87%; f) Tf_2O, CH_2Cl_2, Py, -78 ^oC to 0 ^oC; g) DMF, Et_2NH, 0 ^oC; h) 85% AcOH, 70 ^oC, 60% over three steps; i) NaOMe (30%), MeOH, rt; j) 2 M NaOH aqueous solution, rt; k) methyl ester of corresponding carboxylic acid, MeOH, TEA, Ar, reflux or propionic anhydride, $NaHCO_3$, MeOH, 0 ^oC, 88% for **23**, 100% for **24**, 100% for **25** and 100% for **26**, over three steps; l) $HSCH_2CH_2NH_2{\cdot}HCl$, MeOH, UV (254 nm), rt; m) **8**, DMF, TEA, Ar, rt, 31% for **27**, 47% for **28**, 47% for **29** and 64% for **30**, over two steps; n) PBS, KLH or BSA, rt.

To prepare the *S*-linked STn derivatives with *N*-modifications in sialic acid moiety, the *N*-trifluoroacetyl-protected donor was synthesized by the direct *N*-transacylation of *N*-acetyl-sialyl donor **21** with trifluoroacetic anhydride (Scheme 3).[67-69] The coupling reaction of the glycosyl donor and triflate **35**[47] afforded the *S*-linked disaccharide **36**. The acetal group in **36** was removed with 85% AcOH to obtain the intermediate **37**, which was followed by the sequential treatment with sodium methoxide in methanol and 2 M sodium hydroxide solution, yielding the corresponding disaccharide **38** in 60% yield, along with the byproduct **39** (30% yield) fromed from the 1,5-lactamization.[70] If 0.2 M sodium hydroxide solution was used instead of 2 M sodium hydroxide solution, the proportion of **39** was increased (50% yield). Finally, when the reagent was changed to saturated sodium hydroxide solution, the desired compound **38** (76% yield) was isolated as the practically only product. Subsequently, following the same procedures as mentioned above, the glycoconjugates **48a-51a** and **48b-51b** were prepared successfully.



Scheme 3. The preparation of glycoconjugates **48a-51a** and **48b-51b**. Reagents and conditions: a) trifluoroacetic anhydride, MeCN, TEA, 135 ^{o}C, sealed tube; b) DMF, Et_2NH, 0 ^{o}C, 55%; c) 85% AcOH, 70 ^{o}C, 89%; d) NaOMe (30%), MeOH, rt; e) saturated NaOH solution, rt, 76%; f) methyl ester of corresponding carboxylic acid, MeOH, TEA, Ar, reflux or propionic anhydride, $NaHCO_3$, MeOH, 0 ^{o}C, 100% for **40**, 100% for **41**, 46% for **42** and 91% for **43**; g) $HSCH_2CH_2NH_2$·HCl, MeOH, UV (254 nm), rt; h) **8**, DMF, TEA, Ar, rt, 68% for **44**, 44% for **45**, 44% for **46** and 55% for **47**, over two steps; i) PBS, KLH or BSA, rt.

As a control, the *S*-linked STn **2**, which was obtained previously,[47] was conjugated to carrier proteins by the same protocol (Scheme 4).



Scheme 4. The preparation of glycoconjugates **53a** and **53b**. Reagents and conditions: a) $HSCH_2CH_2NH_2 \cdot HCl$, MeOH, UV (254 nm), rt; b) **8**, DMF, TEA, Ar, rt, 50%, over two steps; c) PBS, KLH or BSA, rt.

Carbohydrate loading levels of the glycoconjugates were determined by estimating sialic acid content by the resorcinol method and protein content by BCA assay (see the Electronic Supplementary Information for more details). With the glycoconjugates in hand, subsequently, the immunological properties of these conjugates were evaluated in mice. Groups of six female BALB/c mice were vaccinated four times at biweekly intervals with the KLH conjugates. The antibody titers of the mouse sera were determined by ELISA with the plate coated by the corresponding BSA conjugate. As shown in Table 1, all of the *N*-modified *S*-linked STn conjugates stimulated strong antigen-specific immune responses after the 4th immunization, whereas the control **53a** showed the similar behavior. Surprisingly, when the plate was coated by the natural STn-BSA conjugate, most of the *N*-modified glycoconjugate vaccines elicited much higher IgG titers of mouse sera than the

control (but still lower than that elicited by natural STn-KLH). Notably, the IgG titer induced by **13a** was increased by 10 times when compared with that induced by the control **53a**. The results clearly displayed that the cross-reactivity of the "modified antibodies" (antibodies that are induced by modified STn antigens) with natural STn antigen could be increased significantly by *N*-modifications of STn. The IgG titers induced by **16a**, **34a** and **51a** are always lower than that induced by the corresponding fluorine-containing modifications. The superiority of fluorinated modifications which might benefit from the similar atom radius and lipophilicity of fluorine and hydrogen atoms is consistent with our previous report.[36]

Table 1. Immunological results after the 4th immunization with the carbohydrate conjugates.

	anti-modified STn IgG titer	anti-natural STn IgG titer	anti-natural STn IgM titer
natural STn-KLH	--	3,482,058	17,232
13a	3,744,946	230,894	10,445
14a	1,428,168	57,847	<1000
15a	1,790,982	111,022	3860
16a	3,949,102	39,753	17358
31a	2,451,535	100,984	3295
32a	4,288,664	189,194	4339
33a	1,149,064	12,482	7242
34a	1,891,990	32,601	2829
48a	584,710	88,290	1936
49a	943,857	20,000	3220
50a	1,402,12	128,396	7923
51a	3,861,430	14,282	1849
53a	1,493,094	22,617	6929

Conclusions

Several *N*-modified *S*-linked STn antigens, as well as their protein conjugates were designed and synthesized. The immunological properties of these synthetic glycoconjugates were evaluated as anti-tumor vaccines in BALB/c mice, and their effects were compared with that of the *S*-linked STn conjugate **53a**. The experimental results disclosed that the cross-reactivity of the "modified antibodies" with natural STn can be improved obviously by suitable *N*-modifications of the *S*-linked STn antigen. The modified glycoconjugate **13a** that induced the highest anti-natural STn IgG titer, might serve as a promising vaccine candidate. More modifications of TACAs are now in progress in our lab.

Experimental Section

General methods. All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Dichloromethane (DCM) and pyridine were distilled over calcium hydride (CaH_2). Ether was dried and distilled from sodium metal. Acetonitril was dried and distilled from P_2O_5. Methanol was distilled from magnesium. *N*, *N*-Dimethylformamide (DMF) was stirred with P_2O_5 and distilled under reduced pressure. Analytical TLC was performed on silica gel 60-F_{254} precoated on aluminium plates (E. Merck), with detection by fluorescence and/or by staining with acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure below 40 °C (bath). Column chromatography was performed employing Silica Gel 200-300 mesh or 230-400 mesh. Optical rotations were measured on a Hanon P850 automatic polarimeter and are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra were recorded on a Bruker AVANCE III-400, Varian INOVA-500 or Bruke AVANCE III-600 spectrometers at 25 °C. Chemical shifts (in ppm) were referenced to tetramethylsilane (δ = 0 ppm) or residual proton solvent. ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with $CDCl_3$ (δ = 77.00 ppm). Compounds **9-12**, **27-30**, **44-47**, **52** are not stable enough for ^{13}C NMR measurement (hydrolysis in D_2O), so their ^{13}C-NMR data cannot be provided (these compounds are stable when stored in solid state at -80 ^{o}C for a few weeks).

High-resolution mass spectrometry was performed on a Thermo Scientific LTQ Orbitrap Discovery or Waters Xevo G2 Qtof spectrometer.

Allyl *S*-(5-fluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-fluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (4): To a stirred solution of **3** (20.0 mg, 0.027 mmol) in MeOH (2.5 mL) was added 30% NaOMe solution in MeOH (0.02 mL). After being stirred at room temperature for 1 h, the solution was concentrated to dryness. To the residue was then added 2 M NaOH solution (0.7 mL) and allowed to stir under reflux overnight. The solution was neutralized with 1 M HCl solution and concentrated. The residue was redissolved in MeOH (1.3 mL). Under an atmosphere of argon, triethylamine (0.5 mL) and methyl fluoroacetate (0.3 mL) was added. The mixture was then stirred under reflux overnight, and concentrated under reduced pressure. The residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 1:4) and then passed through a column of Dowex®50WX4 ion-exchange resin with H_2O to give **4** (15.0 mg, 93%) as an amorphous white solid. $[\alpha]_D^{25}$ +78.8º (*c* 0.4, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.96-5.79 (m, 1H, C*H*=CH_2), 5.28 (d, 1H, *J* = 17.4 Hz, CH=C*H*$_2$), 5.19 (d, 1H, *J* = 10.3 Hz, CH=C*H*$_2$), 4.86 (brs, 1H, H-1), 4.85 (d, 4H, *J* = 48.0 Hz, C*H*$_2$F), 4.20-4.15 (m, 2H), 4.05-3.84 (m, 5H), 3.83-3.60 (m, 4H), 3.58-3.51 (m, 2H), 2.93-2.80 (m, 2H, H-6), 2.76 (d, 1H, *J* = 9.7 Hz, H-3'e), 1.72 (t, 1H, *J* = 11.9 Hz, H-3'a); ^{13}C NMR (150 MHz, D_2O) δ 174.72, 172.20 (d, *J* = 18.5 Hz), 171.83 (d, *J* = 18.6 Hz), 134.30, 119.15, 96.46, 86.43, 80.53 (d, *J* = 180.75 Hz), 80.45 (d, *J* = 179.7 Hz), 75.13, 72.88, 70.68, 69.94, 69.17, 69.17, 68.74, 68.25, 63.36, 52.03, 50.14, 41.62, 30.57; HRMS (ESI) Anal. Calcd for $C_{22}H_{34}N_2O_{13}F_2SNa$ $[M+Na]^+$: 627.1642, found 627.1634.

Allyl *S*-(5-difluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-difluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (5): The synthetic procedure is the same as that described in the synthesis of **4**. $[\alpha]_D^{25}$ +82.7º (*c* 0.6, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 6.10 (t, 1H, *J* = 53.6 Hz, C*H*F_2),

6.09 (t, 1H, *J* = 53.6 Hz, C*H*F_2), 5.93-5.81 (m, 1H, C*H*=CH_2), 5.27 (dd, 1H, *J* = 1.4 Hz, 17.3 Hz, CH=CH_2), 5.19 (d, 1H, *J* = 10.9 Hz, CH=CH_2), 4.87 (d, 1H, *J* = 3.7 Hz, H-1), 4.21-4.12 (m, 2H), 4.05-3.85 (m, 5H), 3.82-3.63 (m, 4H), 3.57-3.52 (m, 1H), 3.49 (dd, 1H, *J* = 1.5 Hz, 9.0 Hz), 2.94-2.80 (m, 2H, H-6), 2.76 (dd, 1H, *J* = 4.8 Hz, 12.7 Hz, H-3'e), 1.72 (t, 1H, *J* = 12.1 Hz, H-3'a); ^{13}C NMR (150 MHz, D_2O) δ 174.67, 166.27 (t, *J* = 25.4 Hz), 166.06 (t, *J* = 25.8 Hz), 134.27, 119.20, 109.02 (t, *J* = 246.6 Hz), 108.89 (t, *J* = 245.7 Hz), 96.15, 86.45, 74.90, 72.95, 70.67, 69.92, 69.18, 69.13, 68.72, 68.08, 63.35, 52.45, 50.71, 41.59, 30.56; HRMS (ESI) Anal. Calcd for $C_{22}H_{32}N_2O_{13}F_4SNa$ $[M+Na]^+$: 663.1453, found 663.1439.

Allyl *S*-(5-trifluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-trifluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (6): The synthetic procedure is the same as that described in the synthesis of **4**. $[\alpha]_D^{25}$ +51.1° (*c* 0.76, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.97-5.80 (m, 1H, C*H*=CH_2), 5.29 (d, 1H, *J* = 17.4 Hz, CH=CH_2), 5.22 (d, 1H, *J* = 10.4 Hz, CH=CH_2), 4.92 (d, 1H, *J* = 3.3 Hz, H-1), 4.27-4.13 (m, 2H), 4.09-3.89 (m, 5H), 3.82-3.68 (m, 4H), 3.57 (dd, 1H, *J* = 6.7 Hz, 12.0 Hz), 3.50 (d, 1H, *J* = 9.0 Hz), 2.97-2.83 (m, 2H, H-6), 2.79 (dd, 1H, *J* = 4.3 Hz, 12.6 Hz, H-3'e), 1.75 (t, 1H, *J* = 12.1 Hz, H-3'a); ^{13}C NMR (150 MHz, D_2O) δ 174.65, 160.12 (q, *J* = 37.5 Hz), 160.09 (q, *J* = 37.7 Hz), 134.25, 119.23, 116.53 (q, *J* = 284.4 Hz), 116.53 (q, *J* = 284.4 Hz), 95.96, 86.46, 74.71, 73.00, 70.67, 69.94, 69.20, 69.06, 68.77, 67.85, 63.36, 52.96, 51.32, 41.61, 30.57; HRMS (ESI) Anal. Calcd for $C_{22}H_{30}N_2O_{13}F_6SNa$ $[M+Na]^+$: 699.1265, found 699.1270.

Allyl *S*-(5-propionylamino-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-propionylamino-2-deoxy-6-thio-*α*-D-galactopyranoside (7): To a stirred solution of **3** (20.0 mg, 0.027 mmol) in MeOH (2.2 mL) was added 30% NaOMe solution in MeOH (0.02 mL). After being stirred at room temperature for 1 h, the solution was concentrated to dryness. To the residue was then added 2 M NaOH solution (0.7 mL) and allowed to stir under reflux overnight. The solution was

neutralized with 1 M HCl solution and concentrated. The residue was redissolved in MeOH (2.7 mL). Sodium bicarbonate (5.0 mg, 0.059 mmol) and propionic anhydride (20.6 μL，0.16 mmol) was added at 0 ^{o}C. The mixture was then stirred for 0.5 h, and concentrated under reduced pressure. The residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 1:4) to give **7** (15.0 mg, 94%) as an amorphous white solid. $[\alpha]_D^{25}$ +76.8º (*c* 0.44, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.91 (ddd, 1H, *J* = 5.8 Hz, 11.2 Hz, 22.3 Hz, C*H*=CH_2), 5.31 (d, 1H, *J* = 17.2 Hz, CH=CH_2), 5.23 (d, 1H, *J* = 10.4 Hz, CH=CH_2), 4.86 (d, 1H, *J* = 3.5 Hz, H-1), 4.22 (dd, 1H, *J* = 5.0 Hz, 13.1 Hz), 4.09 (dd, 1H, *J* = 3.6 Hz, 11.1 Hz), 4.06-3.93 (m, 3H), 3.87 (dd, 1H, *J* = 3.0 Hz, 11.1 Hz), 3.85-3.73 (m, 3H), 3.71-3.63 (m, 1H), 3.59 (dd, 1H, *J* = 5.9 Hz, 11.8 Hz), 3.55-3.50 (m, 2H), 2.95-2.82 (m, 2H, H-6), 2.78 (dd, 1H, *J* = 4.6 Hz, 12.7 Hz, H-3'e), 2.26 (q, 4H, *J* = 7.6 Hz, CH_3CH_2), 1.73 (t, 1H, *J* = 11.9 Hz, H-3'a), 1.07 (t, 6H, *J* = 7.6 Hz, C$H_3$$CH_2$); ^{13}C NMR (100 MHz, D_2O) δ 179.12, 178.63, 174.01, 133.71, 118.37, 95.89, 85.78, 74.89, 72.02, 70.00, 69.26, 68.49, 68.45, 68.26, 67.70, 62.71, 51.66, 49.68, 41.05, 29.87, 29.27, 29.16, 9.60, 9.54; HRMS (ESI) Anal. Calcd for $C_{24}H_{40}N_2O_{13}SNa$ $[M+Na]^+$: 619.2143, found 619.2153.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-fluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-fluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (9): To a solution of **4** (21.0 mg, 0.035 mmol) in degassed MeOH (0.1 mL), cysteamine hydrochloride (13.0 mg, 0.11 mmol) was added and the resulting suspension was irradiated at 254 nm for 4 h. After the mixture was concentrated under reduced pressure, the residue was purified by Sephadex G-10 column chromatography (H_2O). The crude amine was dissolved in DMF (2.8 mL). Triethylamine (7.9 μL, 0.043 mmol) and the *p*-nitrophenyl diester **8** (84.0 mg, 0.23 mmol) were added under argon. After 0.5 h, several drops of acetic acid were added, and the mixture was concentrated. The residue was suspended in 2% acetic acid solution (3.6 mL) and filtered. After being concentrated under reduced pressure, the residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 3:2 with 2% acetic acid) and then Sephadex

G-10 column chromatography (H_2O with 2% acetic acid) to give **9** (11.0 mg, 35%) as a colorless oil. ^{1}H NMR (400 MHz, D_2O) δ 8.29-8.22 (m, 2H, Ar), 7.33-7.25 (m, 2H, Ar), 4.83 (d, 2H, *J* = 46.4 Hz, C*H*$_2$F), 4.82 (d, 2H, *J* = 46.4 Hz, C*H*$_2$F), 4.74 (d, 1H, *J* = 3.8 Hz, H-1), 4.11 (dd, 1H, *J* = 3.7 Hz, 11.0 Hz), 3.94-3.83 (m, 4H), 3.80-3.66 (m, 4H), 3.64 (dd, 1H, *J* = 1.7 Hz, 10.5 Hz), 3.54 (dd, 1H, *J* = 6.6 Hz, 12.3 Hz), 3.50 (dd, 1H, *J* = 1.6 Hz, 9.1 Hz), 3.44-3.35 (m, 1H), 3.32 (t, 2H, *J* = 6.4 Hz), 2.91-2.81 (m, 2H), 2.73 (dd, 1H, *J* = 4.7 Hz, 12.7 Hz), 2.66-2.61 (m, 4H), 2.56 (t, 2H, *J* = 7.1 Hz), 2.24 (t, 2H, *J* = 6.7 Hz), 1.84-1.59 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{53}N_4O_{18}F_2S_2$ $[M+H]^+$: 931.2759, found 931.2772.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-difluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-difluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (10): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.26 (d, 2H, *J* = 9.1 Hz, Ar), 7.30 (d, 2H, *J* = 9.1 Hz, Ar), 6.07 (t, 2H, *J* = 53.6 Hz, C*H*F_2), 4.76 (d, 1H, *J* = 3.8 Hz, H-1), 4.09 (dd, 1H, *J* = 3.7 Hz, 11.0 Hz), 3.97-3.83 (m, 4H), 3.82-3.63 (m, 5H), 3.57-3.52 (m, 1H), 3.47 (d, 1H, *J* = 8.9 Hz), 3.44-3.35 (m, 1H), 3.33 (t, 2H, *J* = 6.5 Hz), 2.95-2.79 (m, 2H), 2.74 (dd, 1H, *J* = 4.7 Hz, 12.8 Hz), 2.67-2.61 (m, 4H), 2.56 (t, 2H, *J* = 6.8 Hz), 2.25 (t, 2H, *J* = 6.6 Hz), 1.80-1.60 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{51}N_4O_{18}F_4S_2$ $[M+H]^+$: 967.2570, found 967.2562.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-trifluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-trifluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (11): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.17 (d, 2H, *J* = 8.6 Hz, Ar), 7.23 (d, 2H, *J* = 8.6 Hz, Ar), 4.80 (d, 1H, *J* = 3.3 Hz, H-1), 4.13 (dd, 1H, *J* = 3.1 Hz, 10.1 Hz), 3.97-3.89 (m, 4H), 3.81-3.70 (m, 5H), 3.57 (dd, 1H, *J* = 7.1 Hz, 12.6 Hz), 3.48 (d, 1H, *J* = 9.0 Hz), 3.39-3.32 (m, 3H), 3.01-2.82 (m, 2H), 2.77 (d, 1H, *J* = 8.1 Hz), 2.61-2.55 (m, 6H), 2.23 (brs, 2H), 1.84-1.53 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{49}N_4O_{18}F_6S_2$ $[M+H]^+$: 1003.2382, found 1003.2373.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-propionylamino-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-propionylamino-2-deoxy-6-thio-*α*-D-galactopyranoside (12): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 7.98 (d, 2H, *J* = 8.6 Hz, Ar), 7.09 (d, 2H, *J* = 8.5 Hz, Ar), 4.04 (d, 1H, *J* = 11.0 Hz), 3.88 (s, 1H), 3.85-3.51 (m, 9H), 3.46 (d, 1H, *J* = 9.0 Hz), 3.34-3.29 (m, 3H), 3.03-2.79 (m, 2H), 2.71 (d, 1H, *J* = 8.7 Hz), 2.64-2.36 (m, 6H), 2.23-2.18 (m, 6H), 1.74-1.58 (m, 7H), 1.03 (t, 3H, *J* = 8.0 Hz, C*H*$_3$CH$_2$), 1.00 (t, 3H, *J* = 7.6 Hz, C*H*$_3$CH$_2$); HRMS (ESI) Anal. Calcd for $C_{38}H_{59}N_4O_{18}S_2$ $[M+H]^+$: 923.3260, found 923.3237.

Allyl 2-trifluoroacetamido-2-deoxy-6-*O*-(*tert*-butyldimethylsilyl)-*α*-D-galactopyranoside (18): A suspension of D-galactosamine hydrochloride **17** (2.5 g, 11.6 mmol), triethylamine (4.1 mL) and CF_3COOMe (1.5 mL, 12.6 mmol) in MeOH (34.7 mL) was stirred overnight at room temperature. The mixture was concentrated *in vacuo* and the residue was heated under reflux for 0.5 h in 8% HCl solution in ally1 alcohol (28.9 mL). The mixture was filtered through celite, and the filtrate was concentrated *in vacuo*. The resulting oil was dissolved in pyridine (18.6 mL) and *t*-butyldimethylsilyl chloride (1.9 g, 12.6 mmol) was added. After being stirred for 16 h at room temperature, the solvent was removed under reduced pressure, and the resulting oil was redissolved in DCM and washed with a saturated aqueous solution of $NaHCO_3$. The organic phase was separated, dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 2:1) to give **18** (2.3 g, 46%) as a colorless oil. $[\alpha]_D^{25}$ +94.8° (*c* 1.6, DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.53 (d, 1H, *J* = 8.9 Hz, N*H*), 5.91-5.84 (m, 1H, C*H*=CH$_2$), 5.30-5.24 (m, 2H, CH=C*H*$_2$), 4.97 (d, 1H, *J* = 3.7 Hz, H-1), 4.41 (td, 1H, *J* = 3.6 Hz, 9.9 Hz), 4.19 (dd, 1H, *J* = 5.3 Hz, 12.9 Hz, C*H*$_2$CH=CH$_2$), 4.14 (s, 1H), 4.01 (dd, 1H, *J* = 6.3 Hz, 12.9 Hz, C*H*$_2$CH=CH$_2$), 3.94 (d, 2H, *J* = 4.4 Hz), 3.82-3.71 (m, 2H), 3.63 (s, 1H), 2.72-2.70 (m, 1H), 0.92 (s, 9H, ^{t}Bu),

0.12 (s, 6H, ^{t}Bu(C*H*$_3$)$_2$Si); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.01 (q, *J* = 37.0 Hz), 133.10, 118.28, 115.79 (q, *J* = 286.0 Hz), 96.11, 69.84, 69.84, 69.69, 68.43, 63.81, 51.08, 25.76, 18.20, -5.54, -5.57; HRMS (ESI) Anal. Calcd for $C_{17}H_{34}N_2O_6F_3Si$ $[M+NH_4]^+$: 447.2133, found 447.2122.

Allyl 2-trifluoroacetamido-2-deoxy-3,4-*O*-isopropylidene-6-*O*-(*tert*-butyldimethyl-silyl) -*α*-D-galactopyranoside (19): To a solution of compound **18** (1.9 g, 4.43 mmol) in acetonitrile (53.2 mL) was added *α*,*α*-dimethoxypropane (10.8 mL, 88.2 mmol) and DL-10-camphorsulfonic aicd (CSA) (506 mg, 2.22 mmol). After being stirred for 15 min at room temperature, the mixture was diluted with DCM, and washed with brine. The organic phase was separated, dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 20:1) to give **19** (1.1 g, 51%) as a colorless oil. $[\alpha]_D^{25}$ +93.1° (*c* 4.36, DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (d, 1H, *J* = 9.3 Hz, N*H*), 5.90-5.80 (m, 1H, C*H*=CH_2), 5.28-5.22 (m, 2H, CH=C*H*$_2$), 4.83 (d, 1H, *J* = 3.3 Hz, H-1), 4.27-4.14 (m, 3H), 4.11 (dd, 1H, *J* = 4.9 Hz, 8.8 Hz), 4.03 (td, 1H, *J* = 2.1 Hz, 6.5 Hz), 3.97 (dd, 1H, *J* = 6.4 Hz, 12.8 Hz), 3.89 (dd, 1H, *J* = 6.7 Hz, 10.0 Hz), 3.82 (dd, 1H, *J* = 6.6 Hz, 10.0 Hz), 1.55 (s, 3H, (C*H*$_3$)$_2$C), 1.33 (s, 3H, (C*H*$_3$)$_2$C), 0.90 (s, 9H, ^{t}Bu), 0.08 (s, 6H, ^{t}Bu(C*H*$_3$)$_2$Si); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.17 (q, *J* = 37.0 Hz), 132.98, 118.41, 115.78 (q, *J* = 286.2 Hz), 109.84, 95.89, 74.02, 72.26, 68.39, 68.34, 62.19, 51.52, 27.93, 26.39, 25.76, 18.20, -5.42, -5.55; HRMS (ESI) Anal. Calcd for $C_{20}H_{34}NO_6F_3SiK$ $[M+K]^+$: 508.1734, found 508.1734.

Allyl 2-trifluoroacetamido-2-deoxy-3,4-*O*-isopropylidene-*α*-D-galactopyranoside (20): To a stirred solution of **19** (58.0 mg, 0.12 mmol) and AcOH (65.8 μL, 1.25 mmol) in THF (3.2 mL) was added TBAF·3H_2O (157.7 mg, 0.50 mmol) at 0 °C under an atmosphere of argon. After being stirred for 4 h at 50 °C, the mixture was concentrated to the half volume and extracted with DCM. The combined extracts were dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was

purified by column chromatography (petroleum ether/ethyl acetate, 2:1) to give **20** (38.0 mg, 87%) as a colorless oil. $[\alpha]_D^{25}$ +113.3º (*c* 1.96, DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (d, 1H, *J* = 8.9 Hz, N*H*), 5.90-5.81 (m, 1H, C*H*=CH_2), 5.30-5.23 (m, 2H, CH=CH_2), 4.89 (d, 1H, *J* = 3.1 Hz, H-1), 4.29-4.14 (m, 4H), 4.12-4.05 (m, 1H), 4.03-3.94 (m, 2H), 3.91-3.81 (m, 1H), 2.32 (dd, 1H, *J* = 2.8 Hz, 8.8 Hz), 1.56 (s, 3H, (CH_3)$_2$C), 1.34 (s, 3H, (CH_3)$_2$C); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.28 (q, J = 38.0 Hz), 132.83, 118.59, 115.76 (q, J = 286.0 Hz), 110.29, 96.10, 74.03, 73.31, 68.66, 67.87, 62.52, 51.38, 27.89, 26.47; HRMS (ESI) Anal. Calcd for $C_{14}H_{20}NO_6F_3Na$ $[M+Na]^+$: 378.1135, found 378.1134.

Allyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2-trifluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (22): To a solution of **20** (492 mg, 1.39 mmol) in DCM (6.0 mL) were added pyridine (0.4 mL, 4.90 mmol) and then triflic anhydride (0.3 mL, 1.77 mmol) dropwise at -78 °C under an atmosphere of argon. After being stirred for 10 min at -78 °C, the reaction mixture was stirred for 1 h at 0 °C using an ice bath. The mixture was washed with 1 M HCl solution and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was passed through a short column of silica gel with petroleum ether/acetone (10:1) to give the triflate as a colorless oil. To a solution of the triflate and **21** (817 mg, 1.49 mmol) in DMF (3.7 mL) was added diethylamine (1.8 mL) at 0 °C under an atmosphere of argon. After being stirred for 2 h, the diethylamine was removed *in vacuo*, and the residue was diluted with ethyl acetate, washed with 1 M HCl solution, H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/acetone, 2:1) and followed by column chromatography (DCM/methanol, 40:1). The resulting disaccharide was then stirred with 85% acetic acid (2.7 mL) at 70 °C overnight and then concentrated under reduced pressure. The crude product was purified by column chromatography (petroleum ether/acetone, 2:1) to give **22** (678 mg, 60%) as an amorphous white solid. $[\alpha]_D^{25}$ +66.2º (*c* 1.96, DCM); ^{1}H NMR (600

MHz, $CDCl_3$) δ 6.66 (d, 1H, *J* = 9.1 Hz, N*H*), 5.87 (ddt, 1H, *J* = 5.7 Hz, 10.6 Hz, 16.6 Hz, C*H*=CH_2), 5.45-5.41 (m, 2H), 5.32 (dd, 1H, *J* = 2.1 Hz, 8.7 Hz), 5.29 (dd, 1H, *J* = 1.5 Hz, 17.2 Hz), 5.23 (dd, 1H, *J* = 1.1 Hz, 10.4 Hz), 4.94-4.87 (m, 2H), 4.36 (dd, 1H, *J* = 2.7 Hz, 12.4 Hz), 4.32 (td, 1H, *J* = 3.7 Hz, 10.0 Hz), 4.20 (dd, 1H, *J* = 5.3 Hz, 13.0 Hz), 4.07-3.96 (m, 3H), 3.92 (d, 1H, *J* = 2.6 Hz), 3.85-3.75 (m, 6H), 3.68 (dd, 1H, *J* = 1.9 Hz, 9.9 Hz), 3.11 (dd, 1H, *J* = 10.0 Hz, 14.0 Hz), 3.13-3.09 (m, 1H), 2.88 (dd, 1H, *J* = 2.6 Hz, 14.0 Hz), 2.74 (dd, 1H, *J* = 4.6 Hz, 12.7 Hz, H-3'e), 2.18 (s, 3H, Ac), 2.15 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.01 (t, 1H, *J* = 12.1 Hz, H-3'a), 1.88 (s, 3H, Ac); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.18, 171.09, 170.88, 170.35, 170.16, 168.80, 157.66 (q, *J* = 36.9 Hz), 133.07, 118.15, 115.76 (q, *J* = 286.2 Hz), 95.72, 82.17, 73.84, 70.87, 70.33, 69.42, 68.85, 68.46, 68.13, 67.08, 62.37, 53.05, 50.83, 49.23, 37.60, 29.97, 23.07, 21.30, 20.77, 20.77, 20.72; HRMS (ESI) Anal. Calcd for $C_{31}H_{43}N_2O_{17}F_3SNa$ $[M+Na]^+$: 827.2127, found 827.2119.

Allyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-fluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (23): To a stirred solution of **22** (25.0 mg, 0.031 mmol) in MeOH (1.5 mL) was added 30% NaOMe solution in MeOH (0.02 mL). After being stirred at room temperature for 1 h, the solution was concentrated to dryness. The residue was then added 2 M NaOH solution (0.7 mL) and allowed to stir at room temperature for 0.5 h. The solution was neutralized with 1 M HCl solution and concentrated. The residue was redissolved in MeOH (1.0 mL). Under an atmosphere of argon, triethylamine (0.4 mL) and methyl fluoroacetate (0.2 mL) was added. The mixture was then stirred under reflux overnight, and concentrated under reduced pressure. The residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 1:4) and then passed through a column of Dowex®50WX4 ion-exchange resin with H_2O to give **23** (16.0 mg, 88%) as an amorphous white solid. $[\alpha]_D^{25}$ +95.8° (*c* 0.48, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.96-5.84 (m, 1H, C*H*=CH_2), 5.30 (dd, 1H, *J* = 1.5 Hz, 17.3 Hz, CH=C*H*$_2$), 5.22 (d, 1H, *J* = 10.4 Hz, CH=C*H*$_2$), 4.87 (d, 2H, *J* = 46.0 Hz, C*H*$_2$F), 4.88 (d, 1H, *J* =

3.7 Hz, H-1), 4.25-4.15 (m, 2H), 4.04 (d, 1H, *J* = 3.0 Hz), 4.02-3.92 (m, 3H), 3.82-3.73 (m, 3H), 3.69-3.54 (m, 2H), 3.52 (d, 2H, *J* = 9.4 Hz), 2.94-2.81 (m, 2H, H-6), 2.76 (dd, 1H, *J* = 4.7 Hz, 12.7 Hz, H-3'e), 1.97 (s, 3H, Ac), 1.71 (dd, 1H, *J* = 11.5 Hz, 12.4 Hz, H-3'a); ^{13}C NMR (150 MHz, D_2O) δ 175.80, 174.72, 171.83 (d, *J* = 18.5 Hz), 134.31, 119.15, 96.46, 86.42, 80.45 (d, *J* = 179.6), 75.56, 72.74, 70.70, 69.91, 69.30, 69.17, 68.85, 68.25, 63.37, 52.44, 50.14, 41.62, 30.55, 22.74; HRMS (ESI) Anal. Calcd for $C_{22}H_{35}N_2O_{13}FSNa$ $[M+Na]^+$: 609.1736, found 609.1721.

Allyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-difluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (24):

The synthetic procedure is the same as that described in the synthesis of **23**. [α]$_D^{25}$ +96.6° (*c* 1.04, MeOH); ^{1}H NMR (600 MHz, D_2O) δ 6.09 (t, 1H, *J* = 53.6 Hz, C*H*F_2), 5.92-5.81 (m, 1H, C*H*=CH_2), 5.26 (dd, 1H, *J* = 1.2 Hz, 17.3 Hz, CH=CH_2), 5.18 (d, 1H, *J* = 10.5 Hz, CH=CH_2), 4.87 (d, 1H, *J* = 3.7 Hz, H-1), 4.19 (dd, 1H, *J* = 5.1 Hz, 13.1 Hz), 4.14 (dd, 1H, *J* = 3.7 Hz, 11.1 Hz), 4.02 (d, 1H, *J* = 3.1 Hz), 3.98 (t, 1H, *J* = 7.1 Hz), 3.96-3.91 (m, 2H), 3.78 (dd, 1H, *J* = 2.3 Hz, 11.9 Hz), 3.74-3.71 (m, 2H), 3.64-3.58 (m, 1H), 3.55 (dd, 1H, *J* = 6.2 Hz, 11.9 Hz), 3.49 (d, 2H, *J* = 9.5 Hz), 2.90-2.80 (m, 2H, H-6), 2.74 (dd, 1H, *J* = 4.8 Hz, 12.7 Hz, H-3'e), 1.95 (s, 3H, Ac), 1.69 (t, 1H, *J* = 12.0 Hz, H-3'a); ^{13}C NMR (150 MHz, D_2O) δ 175.81, 174.72, 166.06 (t, *J* = 25.8 Hz), 134.28, 119.20, 108.89 (t, *J* = 245.7 Hz), 96.13, 86.43, 75.57, 72.75, 70.71, 69.87, 69.29, 69.18, 68.87, 68.08, 63.40, 52.44, 50.70, 41.62, 30.53, 22.75; HRMS (ESI) Anal. Calcd for $C_{22}H_{35}N_2O_{13}F_2S$ $[M+H]^+$: 605.1822, found 605.1827.

Allyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-trifluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (25):

The synthetic procedure is the same as that described in the synthesis of **23**. [α]$_D^{25}$ +106.7° (*c* 0.72, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.88 (dddd, 1H, *J* = 5.2 Hz, 6.6 Hz, 10.4 Hz, 17.1 Hz, C*H*=CH_2), 5.29 (dd, 1H, *J* = 1.5 Hz, 17.3 Hz, CH=CH_2), 5.21 (d, 1H, *J* = 10.4 Hz, CH=CH_2), 4.91 (d, 1H, *J* = 3.7 Hz, H-1), 4.26-4.13 (m, 2H), 4.05

(d, 1H, *J* = 3.1 Hz), 4.03-3.94 (m, 3H), 3.84-3.71 (m, 3H), 3.69-3.48 (m, 4H), 2.90 (dd, 1H, *J* = 5.5 Hz, 11.8 Hz, H-6a), 2.85 (dd, 1H, *J* = 4.4 Hz, 11.9 Hz, H-6b), 2.76 (dd, 1H, *J* = 4.7 Hz, 12.7 Hz, H-3'e), 1.97 (s, 3H, Ac), 1.71 (dd, 1H, *J* = 11.4 Hz, 12.6 Hz, H-3'a); ^{13}C NMR (100 MHz, D_2O) δ 175.09, 173.97, 159.34 (q, *J* = 37.6 Hz), 133.55, 118.48, 115.79 (q, *J* = 284.3 Hz), 95.24, 85.72, 74.84, 72.02, 70.01, 69.15, 68.56, 68.48, 68.20, 67.13, 62.72, 51.73, 50.60, 40.91, 29.79, 22.02; HRMS (ESI) Anal. Calcd for $C_{22}H_{34}N_2O_{13}F_3S$ $[M+H]^+$: 623.1728, found 623.1722.

Allyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-propionylamino-2-deoxy-6-thio-*α*-D-galactopyranoside (26): To a stirred solution of **22** (20.0 mg, 0.025 mmol) in MeOH (1.2 mL) was added 30% NaOMe solution in MeOH (0.02 mL). After being stirred at room temperature for 1 h, the solution was concentrated to dryness. The residue was then added 2 M NaOH solution (0.6 mL) and allowed to stir at room temperature for 0.5 h. The solution was neutralized with 1 M HCl solution and concentrated. The residue was redissolved in MeOH (1.7 mL). Sodium bicarbonate (5.0 mg, 0.059 mmol) and propionic anhydride (12.8 μL，0.099 mmol) was added at 0 °C. The mixture was then stirred for 0.5 h, and concentrated under reduced pressure. The residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 1:4) to give **26** (17.0 mg, 100%) as an amorphous white solid. $[\alpha]_D^{25}$ +70.4° (*c* 0.48, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.90 (ddd, 1H, *J* = 5.7 Hz, 10.7 Hz, 22.4 Hz, C*H*=CH_2), 5.30 (d, 1H, *J* = 17.3 Hz, CH=CH_2), 5.23 (d, 1H, *J* = 10.4 Hz, CH=CH_2), 4.85 (d, 1H, *J* = 3.6 Hz, H-1), 4.20 (dd, 1H, *J* = 5.1 Hz, 13.1 Hz), 4.08 (dd, 1H, *J* = 3.7 Hz, 11.1 Hz), 4.04-3.93 (m, 3H), 3.90-3.74 (m, 4H), 3.67 (td, 1H, *J* = 4.7 Hz, 10.8 Hz), 3.63-3.49 (m, 3H), 2.98-2.83 (m, 2H, H-6), 2.77 (dd, 1H, *J* = 4.5 Hz, 12.7 Hz, H-3'e), 2.25 (q, 2H, *J* = 7.6 Hz, CH_3CH_2), 1.99 (s, 3H, Ac), 1.76 (t, 1H, *J* = 12.0 Hz, H-3'a), 1.07 (t, 3H, *J* = 7.6 Hz, C$H_3$$CH_2$); ^{13}C NMR (100 MHz, D_2O) δ 178.64, 175.08, 173.37, 133.64, 118.35, 95.93, 84.87, 74.88, 71.72, 69.82, 69.29, 68.48, 68.30, 68.23, 67.66, 62.79, 51.72, 49.64, 40.61, 29.79, 29.14, 22.08, 9.60; HRMS (ESI) Anal. Calcd for

$C_{23}H_{39}N_2O_{13}S$ $[M+H]^+$: 583.2167, found 583.2181.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-fluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (27): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.10 (d, 2H, *J* = 8.6 Hz, Ar), 7.18 (d, 2H, *J* = 8.6 Hz, Ar), 4.85 (d, 1H, *J* = 46.0 Hz, C*H*$_2$F), 4.84 (d, 1H, *J* = 46.4 Hz, C*H*$_2$F), 4.77 (d, 1H, *J* = 3.2 Hz, H-1), 4.17 (dd, 1H, *J* = 2.6 Hz, 10.9 Hz), 3.99-3.49 (m, 11H), 3.45-3.27 (m, 3H), 3.00-2.88 (m, 2H), 2.74 (d, 1H, *J* = 8.8 Hz), 2.67-2.46 (m, 6H), 2.23 (brs, 2H), 1.98 (s, 3H, Ac), 1.78-1.63 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{53}N_4O_{18}FS_2Na$ $[M+Na]^+$: 935.2673, found 935.2653.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-difluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (28): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.20 (d, 2H, *J* = 7.3 Hz, Ar), 7.25 (d, 2H, *J* = 7.8 Hz, Ar), 6.09 (t, 1H, *J* = 53.5 Hz, C*H*F_2), 4.76 (brs, 1H, H-1), 4.11 (d, 1H, *J* = 10.1 Hz), 3.97-3.47 (m, 11H), 3.38-3.32 (m, 3H), 2.94-2.85 (m, 2H), 2.73 (d, 1H, *J* = 10.0 Hz), 2.62-2.55 (m, 6H), 2.24 (brs, 2H), 1.96 (s, 3H, Ac), 1.88-1.51 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{52}N_4O_{18}F_2S_2Na$ $[M+Na]^+$: 953.2578, found 953.2596.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-trifluoroacetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (29): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.02 (d, 2H, *J* = 8.5 Hz, Ar), 7.11 (d, 2H, *J* = 8.6 Hz, Ar), 4.80 (brs, 1H, H-1), 4.14 (d, 1H, *J* = 9.5 Hz), 4.01-3.48 (m, 11H), 3.37-3.30 (m, 3H), 2.99-2.87 (m, 2H), 2.75 (d, 1H, *J* = 7.3 Hz), 2.57-2.52 (m, 6H), 2.19 (brs, 2H), 1.99 (s, 3H, Ac), 1.77-1.60 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{51}N_4O_{18}F_3S_2Na$ $[M+Na]^+$: 971.2484, found 971.2512.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl

***S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-propionylamino-2-deoxy-6-thio-*α*-D-galactopyranoside (30):** The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.06 (d, 2H, *J* = 8.8 Hz, Ar), 7.16 (d, 2H, *J* = 8.7 Hz, Ar), 4.75 (d, 1H, *J* = 2.7 Hz, H-1), 4.09 (dd, 1H, *J* = 2.3 Hz, 11.0 Hz), 3.93 (s, 1H), 3.90-3.51 (m, 10H), 3.40-3.34 (m, 3H), 3.01-2.90 (m, 2H), 2.76 (d, 1H, *J* = 8.3 Hz), 2.71-2.46 (m, 6H), 2.25-2.23 (m, 4H), 2.01 (s, 3H, Ac), 1.79-1.64 (m, 7H), 1.05 (t, 3H, *J* = 7.5 Hz, C*H*$_3$CH$_2$); HRMS (ESI) Anal. Calcd for $C_{37}H_{57}N_4O_{18}S_2$ $[M+H]^+$: 909.3104, found 909.3105.

Allyl *S*-(methyl 5-trifluoroacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-6-thio-*α*-D-galactopyranoside (36): To a stirred solution of **21** (2.8 g, 5.16 mmol) in acetonitrile (16 mL) were added triethylamine (4.3 mL, 31.0 mmol) and trifluoroacetic anhydride (2.2 mL, 15.5 mmol). The mixture was stirred at 135 ^{o}C for 5 min in a sealed tube. Then, methanol (6 mL) was added and the reaction mixture was stirred at 0 ^{o}C for 5 min. At this time the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography (petroleum ether/acetone, 3:1) to give the thiosialoside. To a solution of the thiosialoside and triflate **35** (1.4 g, 3.18 mmol) in DMF (9.6 mL) was added diethylamine (4.6 mL) at 0 °C under an atmosphere of argon. After being stirred for 2 h, the diethylamine was removed *in vacuo*, and the residue was diluted with ethyl acetate, washed with 1 M HCl solution, H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/acetone, 2:1) to give **36** (1.5 g, 55%) as an amorphous white solid. $[\alpha]_D^{25}$ +91.3º (*c* 3.16, DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, 1H, *J* = 8.6 Hz, N*H*), 5.95-5.79 (m, 1H, C*H*=CH$_2$), 5.66 (d, 1H, *J* = 9.4 Hz, N*H*), 5.39 (ddd, 1H, *J* = 2.5 Hz, 5.0 Hz, 7.7 Hz), 5.30 (s, 1H), 5.27 (dd, 1H, *J* = 1.4 Hz, 8.5 Hz), 5.22 (dd, 1H, *J* = 1.1 Hz, 10.4 Hz), 5.04 (td, 1H, *J* = 4.7 Hz, 11.6 Hz), 4.78 (d, 1H, *J* = 3.4 Hz, H-1), 4.32 (dd, 1H, *J* = 2.4 Hz, 12.5 Hz),

4.24 (td, 1H, *J* = 3.5 Hz, 9.2 Hz), 4.21-4.12 (m, 3H), 4.07-3.91 (m, 4H), 3.83-3.79 (m, 4H), 3.15 (dd, 1H, *J* = 4.1 Hz, 14.3 Hz, H-6a), 3.03 (dd, 1H, *J* = 9.2 Hz, 14.3 Hz, H-6b), 2.81 (dd, 1H, *J* = 4.7 Hz, 12.8 Hz, H-3'e), 2.15 (s, 3H, Ac), 2.14 (s, 3H, Ac), 2.02 (s, 6H, Ac), 2.02-1.96 (m, 4H, Ac, H-3'a), 1.56 (s, 3H, $(CH_3)_2C$), 1.38 (s, 3H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.70, 170.63, 170.13, 169.97, 169.92, 168.76, 157.53 (q, *J* = 37.5 Hz), 133.44, 117.68, 115.48 (q, *J* = 286.2 Hz), 109.78, 96.74, 82.41, 74.56, 73.78, 73.19, 68.85, 68.65, 68.15, 67.40, 67.16, 61.94, 53.12, 50.29, 50.16, 37.77, 29.59, 27.95, 26.43, 23.38, 21.16, 20.64, 20.62, 20.54; HRMS (ESI) Anal. Calcd for $C_{34}H_{47}N_2O_{17}F_3SNa$ $[M+Na]^+$: 867.2440, found 867.2440.

Allyl *S*-(methyl 5-trifluoroacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (37): Compound **36** (1.2 g, 1.42 mmol) was stirred with 85% acetic acid (4.5 mL) at 70 °C overnight and then concentrated under reduced pressure. The crude product was purified by column chromatography (petroleum ether/acetone, 1:2) to give **37** (1.0 g, 89%) as an amorphous white solid. $[\alpha]_D^{25}$ +79.1° (*c* 4.36, DCM); ^1H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, 1H, *J* = 8.1 Hz, N*H*), 6.08 (d, 1H, *J* = 8.8 Hz, N*H*), 5.86 (ddd, 1H, *J* = 5.6 Hz, 10.7 Hz, 22.6 Hz, C*H*=CH_2), 5.38-5.31 (m, 1H), 5.31-5.22 (m, 2H), 5.18 (dd, 1H, *J* = 1.2 Hz, 10.5 Hz), 4.97 (td, 1H, *J* = 5.2 Hz, 11.0 Hz), 4.79 (d, 1H, *J* = 3.6 Hz, H-1), 4.35 (d, 1H, *J* = 10.5 Hz), 4.31-4.22 (m, 1H), 4.17 (dd, 1H, *J* = 5.1 Hz, 13.0 Hz), 4.13-3.89 (m, 4H), 3.86-3.51 (m, 8H), 3.04 (dd, 1H, *J* = 9.6 Hz, 13.8 Hz), 2.90 (d, 1H, *J* = 11.0 Hz), 2.75 (dd, 1H, *J* = 4.3 Hz, 12.5 Hz, H-3'e), 2.12 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.99-1.92 (m, 4H, Ac, H-3'a); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.98, 171.12, 170.98, 170.77, 169.84, 168.70, 157.59 (q, *J* = 37.4 Hz), 133.50, 117.67, 115.52 (q, *J* = 286.1 Hz), 96.44, 82.58, 73.41, 70.47, 70.30, 70.06, 69.34, 69.06, 67.96, 67.21, 62.25, 53.14, 50.13, 49.74, 37.63, 29.94, 23.15, 21.16, 20.65, 20.55, 20.51; HRMS (ESI) Anal. Calcd for $C_{31}H_{43}N_2O_{17}F_3SNa$ $[M+Na]^+$: 827.2127, found 827.2104.

Allyl *S*-(5-amido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic

acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (38): To a stirred solution of **37** (20.0 mg, 0.025 mmol) in MeOH (1.0 mL) was added 30% NaOMe solution in MeOH (0.02 mL). After being stirred at room temperature for 1 h, the solution was concentrated to dryness. The residue was then added saturated NaOH solution (0.5 mL) and allowed to stir at room temperature for 0.5 h. The solution was neutralized with 1 M HCl solution and concentrated. The residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 1:9) to give **38** (10.0 mg, 76%) as an amorphous white solid. $[\alpha]_D^{25}$ +48.9º (*c* 0.64, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.95 (ddd, 1H, *J* = 5.8 Hz, 11.0 Hz, 22.3 Hz, C*H*=CH_2), 5.34 (dd, 1H, *J* = 1.4 Hz, 17.3 Hz, CH=C*H*$_2$), 5.27 (d, 1H, *J* = 10.4 Hz, CH=C*H*$_2$), 4.89 (d, 1H, *J* = 3.7 Hz, H-1), 4.23 (dd, 1H, *J* = 5.1 Hz, 13.1 Hz), 4.12 (dd, 1H, *J* = 3.7 Hz, 11.1 Hz), 4.07-3.96 (m, 3H), 3.94-3.85 (m, 4H), 3.84-3.68 (m, 3H), 3.26 (t, 1H, *J* = 10.2 Hz, H-5'), 3.01-2.89 (m, 2H, H-6), 2.86 (dd, 1H, *J* = 4.8 Hz, 12.7 Hz, H-3'e), 2.02 (s, 3H, Ac), 1.81 (t, 1H, *J* = 12.1 Hz, H-3'a); ^{13}C NMR (100 MHz, D_2O) δ 174.65, 173.50, 133.69, 118.28, 95.92, 85.73, 73.34, 71.89, 69.79, 69.44, 68.44, 67.85, 67.75, 67.05, 62.30, 52.24, 49.78, 40.91, 29.94, 21.98; HRMS (ESI) Anal. Calcd for $C_{20}H_{35}N_2O_{12}S$ $[M+H]^+$: 527.1905, found 527.1897.

The 1,5-lactam of compound 38 (39): To a stirred solution of **37** (20.0 mg, 0.025 mmol) in MeOH (1.0 mL) was added 30% NaOMe solution in MeOH (0.02 mL). After being stirred at room temperature for 1 h, the solution was concentrated to dryness. The residue was then added 0.2 M NaOH solution (0.5 mL) and allowed to stir at room temperature overnight. The solution was neutralized with 1 M HCl solution and concentrated. The residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 1:9) to give **39** (7.0 mg, 50%) as an amorphous white solid. $[\alpha]_D^{25}$ +87.0º (*c* 0.4, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.93 (ddd, 1H, *J* = 5.8 Hz, 11.0 Hz, 16.4 Hz, C*H*=CH_2), 5.31 (dd, 1H, *J* = 1.6 Hz, 17.3 Hz, CH=C*H*$_2$), 5.22 (dd, 1H, *J* = 1.3 Hz, 10.4 Hz, CH=C*H*$_2$), 4.88 (d, 1H, *J* = 3.8 Hz, H-1), 4.28-4.06 (m, 6H), 4.05-3.98 (m, 2H), 3.87 (dd, 1H, *J* = 3.2 Hz, 11.1 Hz), 3.82-3.79 (m, 1H), 3.79-3.72 (m, 2H), 3.66 (td, 1H, *J* = 2.6 Hz, 7.0 Hz), 3.09 (dd, 1H, *J* = 8.0 Hz, 13.3

Hz, H-6a), 2.93 (dd, 1H, *J* = 6.2 Hz, 13.3 Hz, H-6b), 2.48 (dd, 1H, *J* = 10.5 Hz, 14.3 Hz, H-3'e), 2.18 (dd, 1H, *J* = 4.8 Hz, 14.3 Hz, H-3'a), 2.00 (s, 3H, Ac); ^{13}C NMR (100 MHz, D_2O) δ 174.66, 171.37, 133.78, 118.03, 96.41, 84.71, 78.38, 71.13, 70.79, 70.02, 69.03, 68.74, 67.89, 66.10, 62.30, 52.59, 49.81, 39.53, 28.12, 21.99; HRMS (ESI) Anal. Calcd for $C_{20}H_{33}N_2O_{11}S$ $[M+H]^+$: 509.1800, found 509.1783.

Allyl *S*-(5-fluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (40): To a solution of **38** (8.0 mg, 0.015 mmol) in MeOH (0.5 mL) were added triethylamine (0.2 mL) and methyl fluoroacetate (0.1 mL) under an atmosphere of argon. The mixture was then stirred under reflux overnight, and concentrated under reduced pressure. The residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 1:4) and then passed through a column of Dowex®50WX4 ion-exchange resin with H_2O to give **40** (10.0 mg, 100%) as an amorphous white solid. $[\alpha]_D^{25}$ +73.3º (*c* 0.64, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.91 (dddd, 1H, *J* = 5.2 Hz, 6.5 Hz, 10.5 Hz, 17.1 Hz, C*H*=CH_2), 5.30 (ddd, 1H, *J* = 1.5 Hz, 3.1 Hz, 17.3 Hz, CH=CH_2), 5.23 (dd, 1H, *J* = 10.4, 1.5 Hz, CH=CH_2), 4.88 (d, 2H, *J* = 46.3 Hz, CH_2F), 4.85 (d, 1H, *J* = 3.8 Hz, H-1), 4.21 (ddt, 1H, *J* = 1.4 Hz, 5.1 Hz, 13.2 Hz), 4.08 (dd, 1H, *J* = 3.8 Hz, 11.1 Hz), 4.03 (d, 1H, *J* = 3.1 Hz), 4.01-3.70 (m, 7H), 3.67 (dd, 1H, *J* = 1.9 Hz, 10.4 Hz), 3.63-3.52 (m, 2H), 2.96-2.83 (m, 2H, H-6), 2.79 (dd, 1H, *J* = 4.8 Hz, 12.7 Hz, H-3'e), 1.99 (s, 3H, Ac), 1.75 (dd, 1H, *J* = 11.5 Hz, 12.5 Hz, H-3'a); ^{13}C NMR (100 MHz, D_2O) δ 174.64, 174.00, 171.49 (d, *J* = 18.4 Hz), 133.72, 118.30, 95.89, 85.76, 79.88 (d, *J* = 181.4 Hz), 74.43, 72.15, 69.93, 69.26, 68.46, 68.44, 68.09, 67.75, 62.69, 51.37, 49.77, 40.96, 29.86, 21.99; HRMS (ESI) Anal. Calcd for $C_{22}H_{35}N_2O_{13}FSNa$ $[M+Na]^+$: 609.1736, found 609.1725.

Allyl *S*-(5-difluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (41): The synthetic procedure is the same as that described in the synthesis of **40**. $[\alpha]_D^{25}$ +52.5º

(*c* 0.52, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 6.12 (t, 1H, *J* = 53.6 Hz, C*H*F_2), 5.91 (ddd, 1H, *J* = 5.8 Hz, 11.0 Hz, 22.4 Hz, C*H*=CH_2), 5.30 (d, 1H, *J* = 17.3 Hz, CH=C*H*$_2$), 5.22 (d, 1H, *J* = 10.5 Hz, CH=C*H*$_2$), 4.85 (d, 1H, *J* = 3.7 Hz, H-1), 4.21 (dd, 1H, *J* = 5.1 Hz, 13.1 Hz), 4.08 (dd, 1H, *J* = 3.7 Hz, 11.1 Hz), 4.03 (d, 1H, *J* = 3.0 Hz), 4.01-3.68 (m, 8H), 3.58 (dd, 1H, *J* = 5.8 Hz, 11.4 Hz), 3.52 (d, 1H, *J* = 8.7 Hz), 2.96-2.82 (m, 2H, H-6), 2.79 (dd, 1H, *J* = 4.7 Hz, 12.7 Hz, H-3'e), 1.98 (s, 3H, Ac), 1.75 (t, 1H, *J* = 12.0 Hz, H-3'a); ^{13}C NMR (100 MHz, D_2O) δ 174.64, 173.94, 165.56 (t, *J* = 25.7 Hz), 133.72, 118.29, 108.34 (t, *J* = 247.9 Hz), 95.90, 85.76, 74.20, 72.21, 69.92, 69.28, 68.43, 68.43, 68.06, 67.76, 62.66, 51.79, 49.78, 40.93, 29.88, 21.99; HRMS (ESI) Anal. Calcd for $C_{22}H_{34}N_2O_{13}F_2SNa$ $[M+Na]^+$: 627.1642, found 627.1623.

Allyl *S*-(5-trifluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (42): The synthetic procedure is the same as that described in the synthesis of **40**. $[\alpha]_D^{25}$ +74.7° (*c* 0.36, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.89 (dq, 1H, *J* = 5.7 Hz, 10.8 Hz, C*H*=CH_2), 5.29 (d, 1H, *J* = 17.2 Hz, CH=C*H*$_2$), 5.21 (d, 1H, *J* = 10.6 Hz, CH=C*H*$_2$), 4.83 (d, 1H, *J* = 3.1 Hz, H-1), 4.20 (dd, 1H, *J* = 4.7 Hz, 12.9 Hz), 4.06 (dd, 1H, *J* = 3.4 Hz, 11.0 Hz), 4.03-3.89 (m, 4H), 3.88-3.65 (m, 5H), 3.56 (dd, 1H, *J* = 6.1 Hz, 11.9 Hz), 3.49 (d, 1H, *J* = 9.0 Hz), 2.95-2.81 (m, 2H, H-6), 2.78 (dd, 1H, *J* = 4.5 Hz, 12.7 Hz, H-3'e), 1.97 (s, 3H, Ac), 1.74 (t, 1H, *J* = 12.2 Hz, H-3'a); ^{13}C NMR (100 MHz, D_2O) δ 174.65, 173.92, 159.42 (q, *J* = 36.8 Hz), 133.73, 118.30, 115.84 (q, *J* = 284.6 Hz), 95.92, 85.78, 74.01, 72.26, 69.91, 69.31, 68.45, 68.37, 68.11, 67.77, 62.65, 52.33, 49.78, 40.95, 29.90, 21.99; HRMS (ESI) Anal. Calcd for $C_{22}H_{33}N_2O_{13}F_3SNa$ $[M+Na]^+$: 645.1548, found 645.1531.

Allyl *S*-(5-propionylamino-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (43): To a solution of **38** (7.0 mg, 0.013 mmol) in MeOH (0.9 mL) were added sodium

bicarbonate (3 mg, 0.032 mmol) and propionic anhydride (6.8 μL，0.053 mmol) at 0 ^{o}C. The mixture was then stirred for 0.5 h, and concentrated under reduced pressure. The residue was purified by C-18 reversed-phase column chromatography (CH_3OH/H_2O, 1:4) to give **43** (7.0 mg, 91%) as an amorphous white solid. $[\alpha]_D^{25}$ +121.8^{o} (*c* 0.44, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 5.90 (ddd, 1H, *J* = 5.8 Hz, 11.0 Hz, 22.4 Hz, C*H*=CH_2), 5.30 (dd, 1H, *J* = 1.1 Hz, 17.3 Hz, CH=C*H*$_2$), 5.22 (d, 1H, *J* = 10.4 Hz, CH=C*H*$_2$), 4.85 (d, 1H, *J* = 3.7 Hz, H-1), 4.19 (dd, 1H, *J* = 5.2 Hz, 13.0 Hz), 4.08 (dd, 1H, *J* = 3.7 Hz, 11.1 Hz), 4.03-3.92 (m, 3H), 3.87-3.75 (m, 4H), 3.70 (td, 1H, *J* = 4.6 Hz, 10.8 Hz), 3.64-3.56 (m, 2H), 3.49 (d, 1H, *J* = 9.0 Hz), 2.97-2.85 (m, 2H, H-6), 2.77 (dd, 1H, *J* = 4.6 Hz, 12.8 Hz, H-3'e), 2.24 (q, 2H, *J* = 7.6 Hz, CH_3C*H*$_2$), 1.98 (s, 3H, Ac), 1.79 (t, 1H, *J* = 12.4 Hz, H-3'a), 1.06 (t, 3H, *J* = 7.6 Hz, C*H*$_3$$CH_2$); ^{13}C NMR (100 MHz, D_2O) δ 178.95, 174.58, 172.79, 133.54, 118.22, 95.92, 84.00, 74.85, 71.39, 69.63, 69.25, 68.43, 68.23, 67.89, 67.69, 62.77, 51.50, 49.67, 40.32, 29.68, 29.21, 21.89, 9.47; HRMS (ESI) Anal. Calcd for $C_{23}H_{39}N_2O_{13}S$ $[M+H]^+$: 583.2167, found 583.2171.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-fluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (44): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.16 (d, 2H, *J* = 8.5 Hz, Ar), 7.23 (d, 2H, *J* = 8.6 Hz, Ar), 4.88 (d, 2H, *J* = 46.2 Hz, C*H*$_2$F), 4.74 (brs, 1H, H-1), 4.06 (dd, 1H, *J* = 3.0 Hz, 10.8 Hz), 3.98-3.66 (m, 9H), 3.61 (dd, 1H, *J* = 6.0 Hz, 11.7 Hz), 3.54 (d, 1H, *J* = 9.0 Hz), 3.48-3.28 (m, 3H), 3.05-2.83 (m, 2H), 2.77 (d, 1H, *J* = 8.5 Hz), 2.71-2.51 (m, 6H), 2.26 (brs, 2H), 1.97 (s, 3H, Ac), 1.88-1.60 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{54}N_4O_{18}FS_2$ $[M+H]^+$: 913.2853, found 913.2848.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-difluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (45): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400

MHz, D_2O) δ 8.07 (d, 2H, *J* = 9.0 Hz, Ar), 7.15 (d, 2H, *J* = 8.9 Hz, Ar), 6.08 (t, 1H, *J* = 53.6 Hz, C*H*F_2), 4.02 (dd, 1H, *J* = 3.3 Hz, 11.0 Hz), 3.94-3.63 (m, 9H), 3.56 (dd, 1H, *J* = 6.7 Hz, 12.5 Hz), 3.47 (d, 1H, *J* = 8.9 Hz), 3.41-3.25 (m, 3H), 2.99-2.80 (m, 2H), 2.72 (d, 1H, *J* = 8.3 Hz), 2.65-2.47 (m, 6H), 2.20 (brs, 2H), 1.93 (s, 3H, Ac), 1.83-1.52 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{53}N_4O_{18}F_2S_2$ $[M+H]^+$: 931.2759, found 931.2743.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-trifluoroacetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (46): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.28 (d, 2H, *J* = 9.0 Hz, Ar), 7.33 (d, 2H, *J* = 9.0 Hz, Ar), 4.77 (d, 1H, *J* = 3.7 Hz, H-1), 4.07 (dd, 1H, *J* = 3.7 Hz, 11.0 Hz), 4.03-3.68 (m, 9H), 3.60 (dd, 1H, *J* = 6.9 Hz, 12.5 Hz), 3.51 (d, 1H, *J* = 8.6 Hz), 3.48-3.34 (m, 3H), 2.97 (dd, 1H, *J* = 8.9 Hz, 13.6 Hz), 2.90 (dd, 1H, *J* = 4.8 Hz, 13.6 Hz), 2.79 (dd, 1H, *J* = 4.6 Hz, 12.8 Hz), 2.68 (t, 4H, *J* = 5.9 Hz), 2.61 (t, 2H, *J* = 7.0 Hz), 2.30 (t, 2H, *J* = 6.5 Hz), 1.98 (s, 3H, Ac), 1.88-1.64 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{52}N_4O_{18}F_3S_2$ $[M+H]^+$: 949.2665, found 949.2656.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-propionylamino-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (47): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.28 (d, 2H, *J* = 9.1 Hz, Ar), 7.32 (d, 2H, *J* = 9.1 Hz, Ar), 4.75 (d, 1H, *J* = 3.8 Hz, H-1), 4.04 (dd, 1H, *J* = 3.7 Hz, 11.0 Hz), 3.92 (d, 1H, *J* = 3.0 Hz), 3.88 (dd, 1H, *J* = 5.5 Hz, 8.3 Hz), 3.85-3.64 (m, 6H), 3.64-3.52 (m, 2H), 3.49 (d, 1H, *J* = 9.2 Hz), 3.46-3.31 (m, 3H), 2.96-2.85 (m, 2H), 2.75 (dd, 1H, *J* = 4.5 Hz, 12.8 Hz), 2.67 (dd, 4H, *J* = 6.4 Hz, 11.2 Hz), 2.59 (t, 2H, *J* = 7.0 Hz), 2.30-2.21 (m, 4H), 1.96 (s, 3H, Ac), 1.89-1.62 (m, 7H), 1.05 (t, 3H, *J* = 7.6 Hz, C*H*$_3$$CH_2$); HRMS (ESI) Anal. Calcd for $C_{37}H_{57}N_4O_{18}S_2$ $[M+H]^+$: 909.3104, found 909.3111.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosylonic

acid)-(2→6)-2-acetamido-2-deoxy-6-thio-*α*-D-galactopyranoside (52): The synthetic procedure is the same as that described in the synthesis of **9**. ^{1}H NMR (400 MHz, D_2O) δ 8.23 (d, 2H, *J* = 8.9 Hz, Ar), 7.28 (d, 2H, *J* = 8.9 Hz, Ar), 4.01 (dd, 1H, *J* = 3.7 Hz, 11.0 Hz), 3.89 (d, 1H, *J* = 2.7 Hz), 3.86-3.46 (m, 10H), 3.41-3.37 (m, 1H), 3.33 (t, 2H, *J* = 6.4 Hz), 2.95-2.78 (m, 2H), 2.72 (dd, 1H, *J* = 4.6 Hz, 12.7 Hz), 2.64-2.63 (m, 4H), 2.56 (t, 2H, *J* = 7.0 Hz), 2.24 (t, 2H, *J* = 6.4 Hz), 1.94 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.82-1.60 (m, 7H); HRMS (ESI) Anal. Calcd for $C_{36}H_{55}N_4O_{18}S_2$ $[M+H]^+$: 895.2947, found 895.2939.

General procedure for preparation of the KLH conjugates (13a-16a, 31a-34a, 48a-51a and 53a) or BSA conjugates (13b-16b, 31b-34b, 48b-51b and 53b): A solution of the activated disaccharide (**9-12**, **27-30**, **44-47** and **52**) (10.0 mg) and KLH or BSA (10.0 mg) in PBS (pH = 7.6) was tumbled gently overnight and then dialyzed against PBS (6*500 mL) at 4 ^oC. The glycoconjugate was stored at -20 ^oC prior to use.

The carbohydrate loading levels of the glycoconjugates: The carbohydrate loading of each glycoconjugate was calculated according to the equation shown below：Loading of modified STn (%) = content of disaccharide in the sample/ (content of disaccharide in the sample + content of protein in the sample) *100%

Immunization: Groups of six mice (female pathogen-free BALB/c, age 6-8 weeks, from Department of Laboratory Animal Science, Peking University Health Science Center) were immunized four times at 2-week intervals with the glycoconjugates (each containing 2 μg of carbohydrate in PBS). The vaccines were administered intraperitoneally. Mice were bled prior to the initial vaccination, 13 days after the second and the third vaccinations, and 14 days after the fourth vaccination. Blood was clotted to obtain sera and then heat-inactivated 30 min at 56 ^oC.

Enzyme linked immunosorbent assay (ELISA): ELISA plate (Costar type 3590, Corning Inc.) was first coated respectively with 100 μL of BSA conjugates (**13b-16b**, **31b-36b**, **48b-51b** and **53b**) (including 0.02 μg of carbohydrate) overnight at 4 ^oC (0.1 M bicarbonate buffer, pH 9.6). After washed three times with PBST (250 μL/well), microwells were blocked with 3% BSA (100 μL/well) for 1 h at 37 ^oC. After

the plate was washed, corresponding diluted sera were added to microwells (100 μL/well) and incubated for 1 h at 37 ^{o}C. The plate was washed and incubated with 1:5000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (γ-chain specific) or IgM (μ-chain specific) (Southern Biotechnology Associates, Inc., Buckingham, AL) for 1 h at 37 ^{o}C. The plate was washed, developed with *o*-phenylenediamine (OPD) substrate in the dark for 15 min, and then read at 490 nm. The antibody titer was defined as the dilution showing an absorbance of 0.1, after subtracting background. Meanwhile, the anti-natural STn IgG or IgM titers were determined by ELISA with the plate coated by natural STn-BSA conjugate instead.

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Notes and references

1. M. Fukuda, *Cancer Res*. 1996, **56**, 2237-2744.
2. S. Hakomori, Y. M. Zhang, *Chem. Biol*. 1997, **4**, 97-104.
3. R. D. Astronomo, D. R. Burton, *Nat. Rev. Drug Discovery* 2010, **9**, 308-324.
4. S. J. Danishefsky, J. R. Allen, *Angew. Chem. Int. Ed.* 2000, **39**, 836-863.
5. S. J. Keding, S. J. Danishefsky, in *Carbohydrate-based Drug Discovery,* Vol. 1 (Eds: C. H. Wong), WILEY-VCH, Weinheim, 2003, pp. 381-406.
6. O. Ouerfelli, J. D. Warren, R. M. Wilson, S. J. Danishefsky, *Expert. Rev. Vaccines* 2005, **4**, 677-685.
7. T. Buskas, P. Thompson, G. J. Boons, *Chem. Commun*. 2009, 5335-5349.
8. Z. W. Guo, Q. L. Wang, *Curr. Opin. Chem. Biol*. 2009, **13**, 608-617.
9. M. L. Hecht, P. Stallforth, D. V. Silva, A. Adibekian, P. H. Seeberger, *Curr. Opin. Chem. Biol*. 2009, **13**, 354-359.

10. B. Goldman, L. DeFrancesco, *Nat. Biotechnol.* 2009, **27**, 129-139.
11. C.-C. Liu, X.-S. Ye, *Glycoconj. J.* 2012, **29**, 259-271.
12. U. Westerlind, A. Hobel, N. Gaidzik, E. Schmitt, H. Kunz. *Angew. Chem. Int. Ed.* 2008, **47**, 7551–7556.
13. H. J. Jennings, R. Roy, A. Gamian, *J. Immunol.* 1986, **137**, 1708-1713.
14. R. A. Pon, M. Lussier, Q. L. Yang, H. J. Jennings, *J. Exp. Med.* 1997, **185**, 1929-1938.
15. L. M. Krug, G. Ragupathi, K. K. Ng, C. Hood, H. J. Jennings, Z. Guo, M. G. Kris, V. Miller, B. Pizzo, L. Tyson, V. Baez, P. O.Livingston, *Clin. Cancer Res.* 2004, **10**, 916-923.
16. G. Ragupathi, M. Meyers, S. Adluri, L. Howard, C. Musselli, P. O. Livingston, *Int. J. Cancer* 2000, **85**, 659-666.
17. G. Ragupathi, P. O. Livingston, C. Hood, J. Gathuru, S. E. Krown, P. B. Chapman, J. D. Wolchok, L. J. Williams, R. C. Oldfield, W. J. Hwu, *Clin. Cancer Res.* 2003, **9**, 5214-5220.
18. J. R. Rich, D. R. Bundle, *Org. Lett.* 2004, **6**, 897-900.
19. J. R. Rich, W. W. Wakarchuk, D. R. Bundle, *Chem. Eur. J.* 2006, **12**, 845-858.
20. L. Awad, J. Riedner, P. Vogel, *Chem. Eur. J.* 2005, **11**, 3565-3573.
21. L. Awad, R. Madani, A. Gillig, M. Kolympadi, M. Philgren, A. Muhs, C. Gerard, P. Vogel, *Chem. Eur. J.* 2012, **18**, 8578-8582.
22. C. Mersch, S. Wagner, A.Hoffmann-Röder, *Synlett.* 2009, **13**, 2167-2171.
23. S. Wagner, C. Mersch, A. Hoffmann-Röder, *Chem. Eur. J.* 2010, **16**, 7319-7330.
24. M. Johannes, T. Oberbillig, A. Hoffmann-Röder, *Org. Biomol. Chem.* 2011, **9**, 5541-5546.
25. A. Hoffmann-Röder, A. Kaiser, S. Wagner, N. Gaidzik, D. Kowalczyk, U. Westerlind, B. Gerlitzki, E. Schmitt, H. Kunz, *Angew. Chem. Int. Ed.* 2010, **49**, 8498-8503.
26. A. Hoffmann-Röder, M. Johannes, *Chem. Commun.* 2011, **47**, 9903-9905.
27. M. Zheng, X.-S. Ye, *Tetrahedron* 2012, **68**, 1475-1482.
28. S. Zhang, L. A. Walberg, S. Ogata, S. H. Itzkowitz, R. R. Koganty, M. Reddish, S.

S. Gandhi, B. M. Longenecker, K. O. Lloyd, P. O. Livingston, *Cancer Res.* 1995, **55**, 3364-3368.

29. M. Leivonen, S. Nordling, J. Lundin, K. von Boguslawski, C. Haglund, *Oncology* 2001, **61**, 299-305.
30. S. H. Itzkowitz, E. J. Bloom, W. A. Kokal, G. Modin, S. Hakomori, Y. S. Kim, *Cancer* 1990, **66**, 1960-1966.
31. H. Kobayashi, T. Terao, Y. Kawashima, *J. Clin. Oncol.* 1992, **10**, 95-101.
32. J. L. Werther, M. Tatematsu, R. Klein, M. Kurihara, K. Kumagai, P. Llorens, J. G. Neto, C. Bodian, D. Pertsemlidis, T. Yamachika, T. Kitou, S. Itzkowitz, *Int. J. Cancer* 1996, **69**, 193-199.
33. R. S. Bresalier, Y. Niv, J. C. Byrd, Q. Y. Duh, N. W. Toribara, R. W. Rockwell, R. Dahiya, Y. S. Kim, *J. Clin. Invest.* 1991, **87**, 1037-1045.
34. L. A. Holmberg, B. M. Sandmaier, *Expert. Rev. Vaccines* 2004, **3**, 655–663.
35. S. Sahabuddin, T. C. Chang, C. C. Lin, F. D. Jan, H. Y. Hsiao, K. T. Huang, J. H. Chen, J. C. Horng, J. A. Ho, C. C. Lin, *Tetrahedron* 2010, **66**, 7510-7519.
36. F. Yang, X.-J. Zheng, C.-X. Huo, Y. Wang, Y. Zhang, X.-S. Ye, *ACS Chem. Biol.* 2011, **6**, 252-259.
37. F. Peri, J. Jimenez-Barbero, V. Garcia-Aparicio, I. Tvaroska, F. Nicotra, *Chem. Eur. J.* 2004, **10**, 1433-1444.
38. S. Sabesan, S. Neira, Z. Wasserman, *Carbohydr. Res.* 1995, **267**, 239-261.
39. M. J. Kiefel, B. Beisner, S. Bennet, I. D. Holmes, M. von Itzstein, *J. Med. Chem.* 1996, **39**, 1314-1320.
40. A. Liakatos, M. J. Kiefel, F. Fleming, B. Coulson, M. von Itzstein, *Bioorg. Med. Chem.* 2006, **14**, 739-757.
41. X. Wu, T. Lipinski, E. Paszkiewicz, D. R. Bundle, *Chem. Eur. J.* 2008, **14**, 6474-6482.
42. D. R. Bundle, J. R. Rich, S. Jacques, H. N. Yu, M. Nitz, C. C. Ling, *Angew. Chem. Int. Ed.* 2005, **44**, 7725-7729.
43. C. F. Liang, M. C. Yan, T. C. Chang, C. C. Lin, *J. Am. Chem. Soc.* 2009, **131**, 3138-3139.

44. M. O. Contour-Galcera, Y. Ding, C. Ortiz-Mellet, J. Defaye, *Carbohydr. Res.* 1996, **281**, 119-128.

45. Y. Ding, S. S. Alkan, G. Baschang, J. Defaye, *Carbohydr. Res*. 2000, **328**, 71-76.

46. E. Bousquet, A. Spadaro, M. S. Pappalardo, R. Bernardini, R. Romeo, L. Panza, G. Ronsisvalle, *J. Carbohydr. Chem.* 2000, **19**, 527-541.

47. C.-X. Huo, X.-S. Ye, *J. Chin. Pharm. Sci.* 2009, **18**, 214-217.

48. M. Nitz, D. R. Bundle, *J. Org. Chem.* 2001, **66**, 8411-8423.

49. X. Wu, C. C. Ling, D. R. Bundle, *Org. Lett.* 2004, **6**, 4407-4410.

50. C. D. Heidecke, T. K. Lindhorst, *Chem. Eur. J.* 2007, **13**, 9056-9067.

51. X. Wu, T. Lipinski, F. R. Carrel, J. J. Bailey, D. R. Bundle, *Org. Biomol. Chem.* 2007, **5**, 3477-3485.

52. M. Hartmann, A. K. Horst, P. Klemm, T. K. Lindhorst, *Chem. Commun.* 2010, **46**, 330-332.

53. P. B. van Seeventer, J. A. L. M. van Dorst, J. F. Siemerink, J. P. Kamerling, J. F. G. Vliegenthart, *Carbohydr. Res.* 1997, **300**, 369-373.

54. M. Dubber, T. K. Lindhorst, *Carbohydr. Res.* 1998, **310**, 35-41.

55. S. Wittrock, T. Becker, H. Kunz, *Angew. Chem. Int. Ed.* 2007, **46**, 5226-5230.

56. M. O. Trollsas, D. C. Gale, Y. Wang, US patent, 015366. 2007-07-02.

57. C. C. Chu, H. Song, US patent, 084934. 2008-11-26.

58. K. Guo, C. C. Chu, E. Chkhaidze, R. Katsarava, *J. Polym. Sci. A-Polym. Chem*. 2005, **43**, 1463-1477.

59. R. Katsarava, V. Beridze, N. Arabuli, D. Kharadze, C. C. Chu, C. Y. Won, *J. Polym. Sci. A-Polym. Chem.* 1999, **37**, 391-407.

60. T. J. Curphey, *J. Org. Chem.* 1979, **44**, 2805-2807.

61. C. Rosenbohm, D. V. Berghe, A. Vlietinck, J. Wengel, *Tetrahedron* 2001, **57**, 6277-6287.

62. M. Shiozaki, Y. Kobayashi, N. Ishida, M. Arai, T. Hiraoka, *Carbohydr. Res.* 1991, **222**, 57-68.

63. G. Garcia-Martin, C. Gasch, A. Gomez-Sanchez, A. Gomez-Sanchez, *Carbohydr. Res.* 1987, **162**, 181-197.

64. B. H. Lipshutz, J. C. Barton, *J. Org. Chem.* 1988, **53**, 4495-4499.

65. A. Ishii, H. Hojo, A. Kobayashi, K. Nakamura, Y. Nakahara, Y. Ito, Y. Nakahara, *Tetrahedron* 2000, **56**, 6235-6243.

66. A. Hasegawa, J. Nakamura, M. Kiso, *J. Carbohydr. Chem.* 1986, **5**, 11-19.

67. P. Rota, P. Allevi, R. Mattina, M. Anastasia, *Org. Biomol. Chem.* 2010, **8**, 3771-3776.

68. P. Allevi, M. Anastasia, M. L. Costa, P. Rota, *Tetrahedron: Asymmetry* 2011, **22**, 338-344.

69. P. Rota, P. Allevi, M. L. Costa, M. Anastasia, *Tetrahedron: Asymmetry* 2010, **21**, 2681-2686.

70. H. Ando, Y. Koike, S. Koizumi, H. Ishida, M. Kiso, *Angew. Chem. Int. Ed.* 2005, **44**, 6759-6763.