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# Chemical and Chemoenzymatic Synthesis of S-Linked Ganglioside Analogues and Their Protein Conjugates for Use as Immunogens

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**Abstract:** Analogues of the tumor-associated gangliosides $GM_3$ and $GM_2$ containing terminal S-linked neuraminic acid residues and an amino terminated, truncated ceramide homologue have been synthesized and conjugated to a protein. The synthesis involved coupling of a S-linked sialyl α(2→3) galactose disaccharide with a glucosyl sphingosine analogue, followed by elaboration and deprotection to give amino-terminated glycosyl ceramide **1**. Glycosyltransferase-catalyzed extension of the trisaccharide **1** provided access to the modified $GM_2$ tetrasaccharide **2** or sulphur-containing $GD_3$ analogue **30**. Owing to their potentially enhanced resistance to endogenous *exo*-glycoside hydrolases and their inherent non-self character, carbohydrate antigens containing non-reducing terminal thioglycosidic linkages may be more immunogenic than *O*-linked antigens and may stimulate the production of antibodies capable of recognizing naturally occurring oligosaccharides. Our initial results suggest that in fact these antigens are viable immunogens and furthermore, that immune sera cross reacts with *O*-gangliosides in the context of a heterologous glycoprotein conjugate.

**Keywords:** carbohydrates · gangliosides · glycoconjugates · oligosaccharides · vaccines

## Introduction

Complex carbohydrates decorate the surfaces of bacteria, parasites, viruses, tumors and numerous other targets for which vaccine development is an important and active area of research.[1–3] Owing to the prominent location of these molecules at the interface of the cell and its surroundings, the generation of a carbohydrate specific immune response is an attractive means of developing therapies to combat the effects of pathogens such as HIV,[4] and malaria,[5] as well as diseases such as cancer.[6,7] The conjugation of defined oligosaccharide epitopes to immunogenic carrier proteins has been advanced as a means to overcome the generally poor immune response to otherwise T-cell independent carbohydrate antigens.[8] The feasibility of this approach has recently been demonstrated in the development of a fully synthetic glycoconjugate vaccine against *Haemophilus influenzae* type b.[9,10] However, in most cases additional, complementary strategies for the generation of carbohydrate specific immune responses remain in demand.

Carbohydrate immunogens and other therapeutics are susceptible to in vivo degradation by glycoside hydrolases. The incorporation of unnatural, hydrolysis resistant carbohydrate analogues into vaccine constructs has been proposed as a means to enhance the bioavailability of the antigen to the immune system, but would be of use only if the resultant immune response were to target the naturally occurring structure.[11,12] To this end, hydrolysis resistant carbohydrate analogues must maintain conformations similar to those of the target oligosaccharides.

Substitution of the glycosidic oxygen atom by sulfur or carbon is a well known method to enhance the stability of the glycosidic linkage towards hydrolysis by either chemical or enzymatic means, and in fact, these molecules have been demonstrated to inhibit the activity of glycosidases and other carbohydrate-binding proteins in some cases.[13–18] It has been demonstrated that while S-glycosides are significantly more flexible about the glycosidic linkage and are consequentially more prone to adopt the high energy *anti* conformations, in many cases they sample similar conformational space to O-glycosides.[19–26] Although S-glycosides, in the form of a thiooligosaccharide–protein conjugate and thioglycopeptide, have been evaluated for their in vitro immu-

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nostimulatory activity,[12,27] we are unaware of any instance in which these molecules have been evaluated as immunogens in vivo.

Many ganglioside glycolipids have been identified as tumor-associated antigens, which as a consequence of their vast over expression on the surface of tumor cells may serve as targets for cancer immunotherapy. It is proposed that tumor-associated ganglioside containing conjugate vaccines will induce anti-ganglioside antibodies that have the potential to effect clearance of circulating tumor cells and thus inhibit further metastasis.[28,29] Several clinical trials have shown promising results in this regard.[30] In particular the tri- to pentasaccharides $GM_3$, $GM_2$, $GD_3$, and $GD_2$ (Figure 1), have received attention for use as immunogens in active immunotherapy against melanoma and other cancers.[30–32]

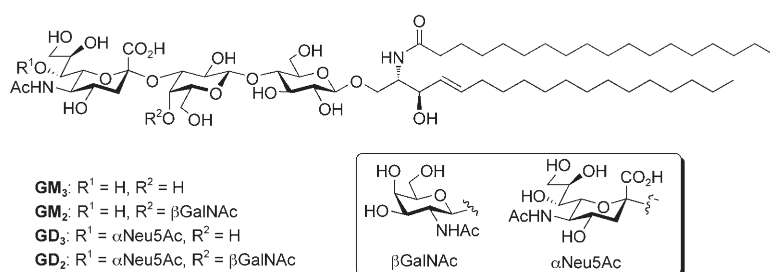


Figure 1. Structures of the tumor associated gangliosides $GM_3$, $GM_2$, $GD_3$, and $GD_2$.

While the structural similarity of O- and S-linked gangliosides is critical to this concept, paradoxically their necessary differences may also serve to identify S-glycosides as nonself antigens and thus further enhance their immunogenicity relative to O-glycosides. Towards these ends we have developed syntheses of sulfur containing analogues (**1** and **2**) of $GM_3$ and $GM_2$ and synthesized the corresponding carbohydrate-protein conjugates for evaluation as immunogens in mice (Figure 2). Only the terminal sialosyl linkage was targeted for stabilization as the majority of glycoside hydrolases function via cleavage of residues at the non-reducing glycan terminus (*exo*-glycosidases). Glycosides of *N*-acetylneuraminic acid are known to be susceptible to enzymatic and acidic hydrolysis,[33] and sialidase levels have been suggested to be up-regulated in the sera of cancer patients.[34–36] The plasma membrane sialidase Neu3 is a ganglioside-specific hydrolase that has been demonstrated to exert activity toward the surface of neighbouring cells, synthetic glycoprotein conjugates, and $GM_3$.[37,38] It should be noted that glycosylation adjacent to the point of sialic acid attachment significantly reduces the susceptibility of this residue to neuraminidase.[39,40] In keeping with other extracellular sialidases, Neu3 does not desialylate ganglioside $GM_2$, which is degraded lysosomally. However, we have constructed S-$GM_2$ in order to investigate the consequence of an adjacent branching residue on the thiosialoside epitope. Our initial results indicate that S-gangliosides conjugated to tetanus toxoid (TT) induce an IgG response in mice and that the immune sera cross react with O-gangliosides.[41]

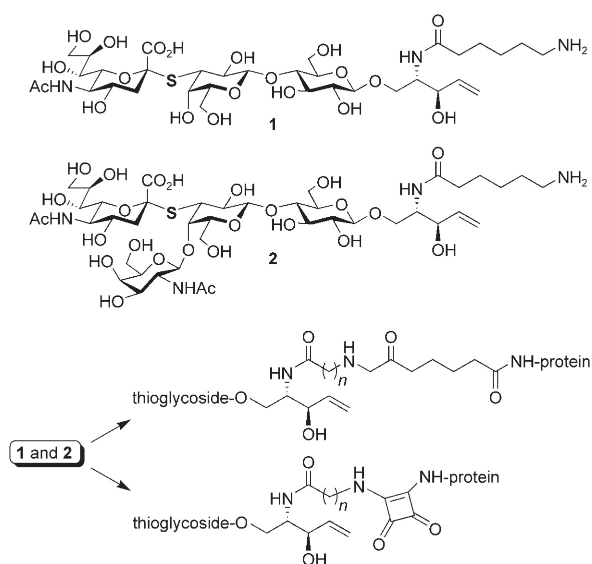


Figure 2. Target S-gangliosides and glycoprotein conjugates.

## Results and Discussion

**Retrosynthesis**: The target neoglycolipids were synthesized from the derivatives outlined in the retrosynthetic Scheme 1a. Modifications to the naturally occurring glycolipids were limited to substitution of the terminal sialosyl glycosidic oxygen by sulfur and use of a truncated ceramide aglycon (**9**) to facilitate coupling to protein and enhance the water solubility of the antigens. We have previously communicated the synthesis of S-$GM_3$ derivatives by a new route involving assembly of the thiosialyllactose trisaccharide followed by coupling with a truncated azidosphingosine and subsequent elaboration to the glycosyl ceramide (Scheme 1b).[42] However, in order to secure fragments of the glycolipids suitable for evaluation of antisera by ELISA, we chose to employ an entirely new synthetic route centred around S-$GM_4$ disaccharide donor **4** and the truncated glucosyl sphingosine acceptor **5**.

Briefly, S-$GM_2$ (**2**) was potentially available from S-$GM_3$ (**1**) by glycosyltransferase catalyzed addition of *N*-acetylgalactosamine. A protecting group strategy was selected that would allow for a chemical synthesis of the S-$GM_2$ tetrasaccharide from S-$GM_3$ in the event that this approach was unsuccessful. Although we have recently demonstrated the use of carbohydrate thiols as acceptors in glycosyltransferase


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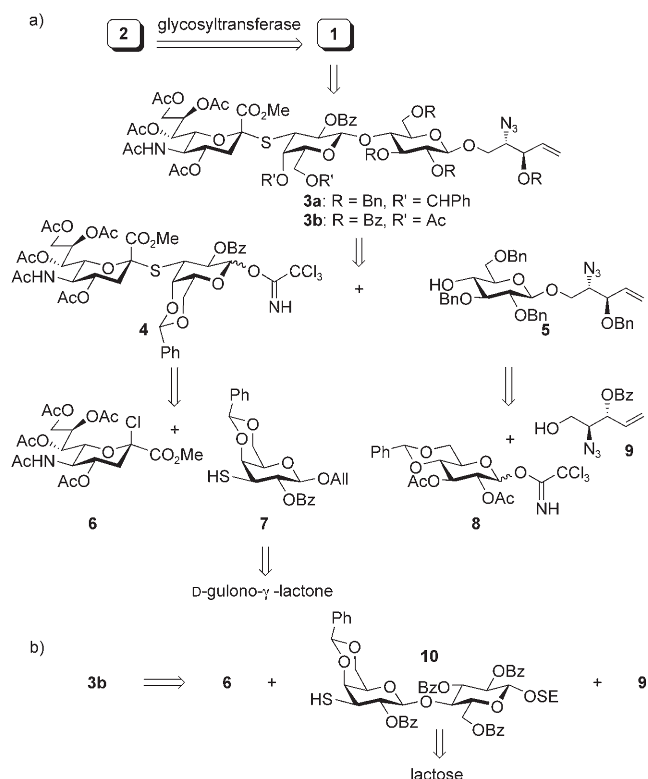
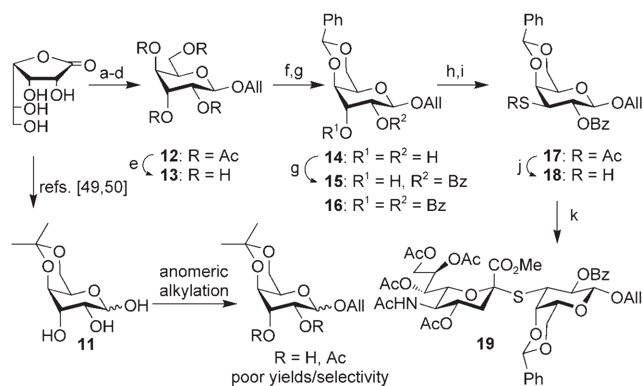


Scheme 1. Retrosynthetic analysis of a) S-linked gangliosides **1** and **2**, and b) protected S-$GM_3$ trisaccharide **3b**.

catalyzed glycosylations, 3′-thiolactosides have proven unsuitable substrates for sialyltransferases that we have studied, necessitating a chemical synthesis of S-$GM_3$.[43] The protected trisaccharide **3a** was constructed via the coupling of the S-$GM_4$ disaccharide **4** with glucoside **5**.

**Synthesis of the S-$GM_4$ disaccharide 19** (Scheme 2): The α-(2→3) linked sialyl galactose disaccharide is a fragment central to all gangliosides, is present as a repeating unit in higher gangliosides, and is common to numerous other glycoconjugates. 3-Thiogalactosides have previously been synthesized from D-gulopyranosides,[14,42,44–46] and D-gulofuranosides.[47] High yielding inversions at C-3 of gulopyranosides require protection of the 4,6-diol as a cyclic acetal and are favoured by use of ester, rather than ether protecting groups at O-2.[42,48] Two synthetic routes to substrates meeting these requirements were identified and are outlined in Scheme 2.

D-Gulose, available on large scale and in excellent yield by reduction of D-gulono-γ-lactone with sodium borohydride,[49] is readily converted to the 4,6-O-isopropylidene protected triol **11**.[50] Unfortunately, attempts to alkylate the anomeric position of the triol or the related 2,3-diacetate by reaction with allyl bromide and base resulted in poor stereoselectivities and moderate yields. Instead, the allyl guloside **13** was prepared, with the expectation of future conversion to a glycosyl donor or elaboration of the terminal olefin for the preparation of glycoconjugates. Subsequent installation of a 4,6-benzylidene acetal with benzaldehyde dimethyl



Scheme 2. Synthesis of protected thiosialosyl galactoside **19**. a) $NaBH_4$, $H_2O$, $H^+$ resin, 0°C; b) $Ac_2O$, pyridine; c) 45% HBr/HOAc, $CH_2Cl_2$, 0°C→RT; d) AllOH, Drierite, $Hg(CN)_2$, $HgBr_2$; e) cat. NaOMe, MeOH, 73% over 3 steps; f) $PhCH(OMe)_2$, DMF/MeCN, cat. *p*TSA·$H_2O$, 73%; g) pyridine, BzCl, $CH_2Cl_2$, 0°C, 94%; h) $Tf_2O$, pyridine, $CH_2Cl_2$, −20→−5°C; i) KSAc, DMF, 50°C, 94% over 2 steps; j) hydrazine acetate, DMF, 98%; k) NaH, DMF, then **6**, Kryptofix 21, 90%.

acetal in a mixture of DMF and acetonitrile containing catalytic acid yielded the diol **14**. When this reaction was conducted by using either solvent alone, small quantities of the diastereomeric benzylidene acetal contaminated the reaction mixture.[51] Regioselective benzoylation at the more reactive equatorial hydroxyl yielded the axial alcohol **15** and a trace of dibenzoate **16**. The alcohol was smoothly converted to the thioester **17** by reaction of the corresponding triflate with potassium thioacetate in DMF. De-*S*-acetylation with hydrazine acetate gave the thiol and small amounts of disulfide. Condensation of the **18** with 2-chlorosialic acid derivative **6** under basic conditions afforded the thiodisaccharide **19** in high yield.[52]

**Synthesis of protected glucosyl sphingosine analogues 5 and 23** (Scheme 3): Traditional approaches to the preparation of glycosyl ceramide–protein conjugates have involved isolation or total synthesis of the glycolipid followed by degradative ozonolysis of the olefin to yield the aldehyde, which is then coupled to protein via reductive amination. Our group has described the use of modified ceramide aglycons derived from an azidosphingosine homologue **9**.[42,53,54] This strategy allows for an abridged synthesis, facilitates glycoconjugate formation, enhances water solubility of the neoglycolipids, and simplifies handling of the chemical intermediates while maintaining the stereochemical integrity and many of the important structural features found in naturally occurring ceramides. Conjugation to protein may be achieved by either elaboration of the terminal alkene, for example via the photoaddition of cysteamine and subsequent reaction of the resulting amine, or by installation of a suitably functionalized *N*-acyl group after reduction of the azide function. We and others have demonstrated that such truncated molecules may be efficiently elaborated to the full-length sphingosines by olefin cross metathesis.[55–58]

Reaction of the glucosyl trichloroacetimidate **8**[59] with the azido alcohol **9** (Scheme 3) afforded the glycoside **20** in high

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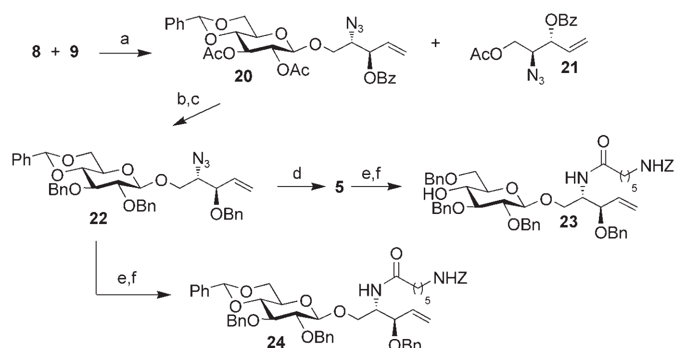
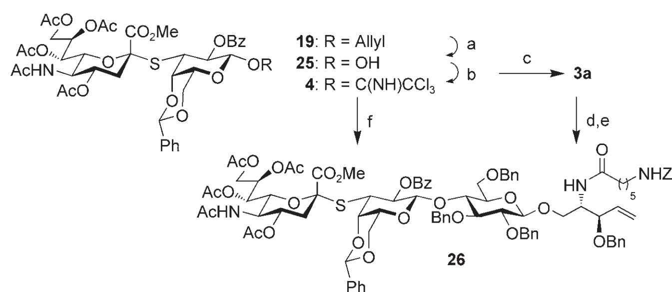


Scheme 3. Construction of protected glucosyl ceramide analogues **5** and **23**. a) 4 Å MS, $CH_2Cl_2$, TMSOTf, −50°C→RT, 90%; b) cat. NaOMe, MeOH; c) NaH, BnBr, DMF, 0°C→RT, 92%; d) 3 Å MS, $NaCNBH_3$, HCl, $Et_2O$, THF, 94%; e) $PPh_3$, pyridine/$H_2O$ 9:1; f) 6-(benzyloxycarbonylamino)hexanoic acid succinimidyl ester, 45°C, 79% for **23**, 85% for **24**.

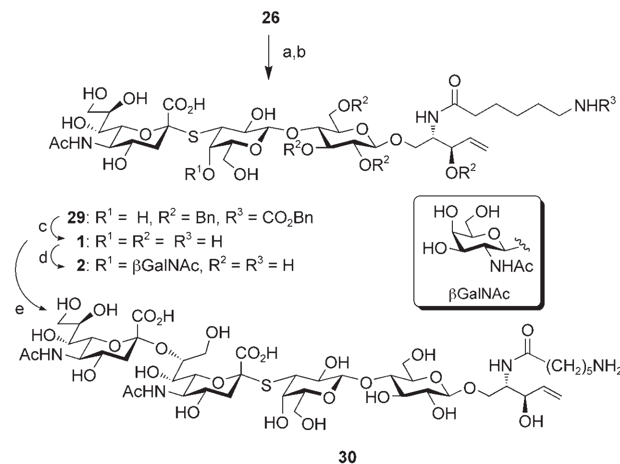


Scheme 4. Synthesis of protected S-$GM_3$ trisaccharide **26**. a) $PdCl_2$, NaOAc, HOAc/$H_2O$ 19:1, ))), <30°C, 81%; b) $K_2CO_3$ or DBU, $Cl_3CCN$, $CH_2Cl_2$, ~70%; c) **5**, 4 Å MS, $BF_3{\cdot}Et_2O$, 0°C→RT, 64%; d) $PPh_3$, pyridine/$H_2O$ 9:1, 50°C; e) 6-(benzyloxy carbonylamino)hexanoic acid succinimidyl ester, 50°C, 90%; f) **23**, 4 Å MS, $BF_3{\cdot}Et_2O$, 0°C→RT, 52%.

yield along with a small amount of the acetylated acceptor **21**. Exchange of ester protecting groups for benzyl ethers was followed by regioselective benzylidene acetal opening to furnished the acceptor **5** and a small amount of benzyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (<2%).[60] Reduction of the azide and *N*-acylation provided the alternative acceptor **23**.

**Coupling of S-$GM_4$ disaccharide and glucosyl sphingosines** (Scheme 4): Removal of the allyl protecting group at the anomeric centre proved problematic. Reaction of **19** with tris(triphenylphosphine) rhodium chloride gave only small amounts of the isomerized product (<10%).[61] A one-pot deprotection was attempted by using palladium chloride in methanol. Under these conditions product decomposition occurred before significant quantities of starting material were consumed. Exchange of methanol for buffered acetic acid[62] gave slightly better yields (~40%), but decomposition predominated. Fortunately, a significant rate enhancement was realized when the reaction mixture was immersed in an ultrasonic cleaning bath (<30°C), and the disaccharide hemiacetal **25** could be isolated as a mixture of isomers in reasonable yield (81%) after only 5 h. Conversion of the hemiacetal to the trichloroacetimidate **4** was effected by using either potassium carbonate or DBU as base.[63] Glycosylation of acceptor **5** with **4** by using boron trifluoride diethyletherate or trimethylsilyl trifluoromethanesulfonate as promoter in dichloromethane gave moderate yields of the trisaccharide **3a**. One-pot reduction of the azide and acylation of the resulting amine with 6-amino-*N*-carboxybenzyl caproic acid NHS ester[64] yielded protected $GM_3$ analogue **26**. Similarly, glycosylation of the *N*-acylated acceptor **23** gave **26** directly, albeit in slightly reduced overall yield.

**Deprotection of S-$GM_3$ and extension to thiotetrasaccharide S-$GM_2$** (Scheme 5/Figure 3): It was envisaged that a chemical synthesis of S-$GM_2$ from **26** could be accomplished following either hydrolysis of the acetal protecting group and selective protection of the resulting primary alcohol **27** or regioselective cleavage of the acetal to give **28** (see Figure 3). Prior to investigation of this route, protected S-$GM_3$ was fully deblocked (**26** → **29** → **1**, Scheme 5) to test as a substrate for glycosylation by a β(1,4) *N*-acetylgalactosaminyl transferase. It is noteworthy that substantial losses of the deprotected trisaccharide **1** were incurred when conventional SepPak or C18 silica gel HPLC columns were employed. This problem was overcome by use of a polymeric reverse phase column from Hamilton (see Experimental Section), or by inclusion of ammonium hydroxide in the mobile phase.



Scheme 5. Deprotection of S-$GM_3$ and enzymatic elaboration to S-$GM_2$ (**2**) and O,S-$GD_3$ (**30**). a) cat. NaOMe, MeOH, 55°C; b) NaOH, MeOH, 40°C, 94%; c) Na, $NH_3$, THF, −78°C, 83%; d) β(1,4) GalNAc transferase, UDP-GlcNAc 4-epimerase, UDP-GlcNAc, 74%; e) α(2,3/8) Neu5Ac transferase, CMP-Neu5Ac, 38%.

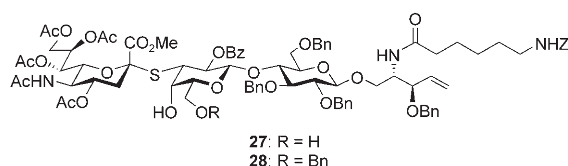


Figure 3. Potential intermediates in the chemical synthesis of S-$GM_2$.

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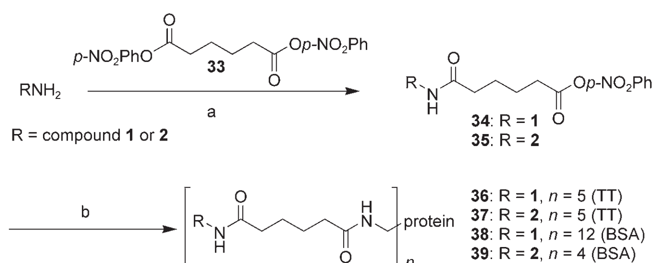
Despite its thioglycosidic linkage we found that **1** served as an acceptor for glycosyltransferase catalyzed elaboration to **2** (Scheme 5). Several examples of a Leloir glycosyltransferase-mediated glycosylation of a thiooligosaccharide acceptor have been reported.[43,65,66] Bacterial transglucosylases,[67–69] as well as sulfotransferases and acyltransferases from *Sinorhizobium meliloti*,[70] have also been shown to utilize thiooligosaccharides as acceptors. The reaction was carried out in the presence of two previously cloned enzymes, a bacterial β(1,4) *N*-acetylgalactosaminyl transferase from *Campylobacter jejeuni* O:36,[71] and a UDP-GlcNAc 4-epimerase[72] from *C. jejuni* NCTC 11168 that catalyzes the conversion of UDP-GlcNAc to the more expensive UDP-GalNAc. Treatment of **1** with a cloned bifunctional α(2,3/8) sialyltransferase[73] and the appropriate sugar nucleotide donor resulted in conversion to the sulfur containing $GD_3$-analogue **30**. Successful glycosylation of the thiooligosaccharide by two different enzymes encourages the conclusion that **1** readily adopts conformations similar to that of O-sialyllactose. With the amino-terminated thioglycosides **1** and **2** in hand, coupling to protein could be undertaken.

**Synthesis of carbohydrate–protein conjugates for immunization and screening of immune sera**: Recently, our lab has obtained high IgG titres against TT–carbohydrate conjugates in rabbits,[74] and monomeric TT was selected as the carrier protein for this study. Diethyl squarate has gained favour as an efficient cross linking agent for the preparation of glycoconjugates.[75] However, work in our group has identified linker specific antibody responses and as a consequence we have discontinued its use for synthesis of immunogens.[74] Instead, we prefer a linear adipic acid derived homobifunctional linker that can be employed for the generation of oligosaccharide–protein conjugates.[76] Conjugation via either squarate or adipate linkers requires reaction of the activated ester with an amine terminated deprotected oligosaccharide such as **1** or **2**, followed by reaction of the product with protein in aqueous solution. Screening antigens were coupled to BSA via diethyl squarate, to enable quantitation of the immune response by ELISA while avoiding measurement of protein or linker specific antibodies.

The conjugation of thiooligosaccharides **1** and **2** to TT and BSA is outlined in Scheme 6. The amines **1** and **2** were treated with an excess of linker **33** in anhydrous DMF containing triethylamine. Following precipitation of the excess coupling agent with 1% acetic acid solution, the activated esters **34** and **35** were purified by HPLC. Coupling with tetanus toxoid and BSA was carried out as previously reported,[76] by using an activated saccharide to protein ration of 30:1 to yield conjugates **36–39**. Incorporation levels are indicated in Scheme 6.

Structures of the amine terminated mono-, di- and oligosaccharides conjugated to BSA with diethyl squarate are outlined in Figure 4. The synthesis of O-$GM_3$ (**40**), O-$GM_2$ (**41**) and lactosyl ceramide (**42**) analogues will be reported elsewhere.[77] The cerebroside analogue **43** was available after dissolving metal reduction of protected glucoside **24**.




Scheme 6. Conjugation of **1** and **2** to tetanus toxoid and bovine serum albumin via adipate linker **33**. a) **33**, DMF, $Et_3N$, 81% for **1**, 56% for **2**; b) tetanus toxoid or bovine serum albumin, phosphate buffered saline, pH 7.2. Average hapten incorporation levels were determined by MALDI-TOF.

Amino terminated $GM_4$ disaccharide **45** was obtained via enzymatic sialylation[78] of 6-azidohexyl β-D-galactopyranoside[79] followed by reduction of the azide **44** with triphenylphosphine. Coupling was achieved by reaction of the amines **1**,**2**,**40–43** and **45** with 0.95 equivalents of diethyl squarate in methanol followed by removal of solvent and reaction with BSA in borate buffer at pH 9.0. Average hapten incorporation as determined by MALDI TOF mass spectrometry is outlined in Table 1.

Table 1. Hapten incorporation for BSA conjugates using diethyl squarate as coupling reagent.

| Amine | Incorporation (*n*) |
|---|---|
| **1** | 5 |
| **2** | 4 |
| **40** | 10 |
| **41** | 6 |
| **42** | 7 |
| **43** | 20 |
| **45** | 6 |

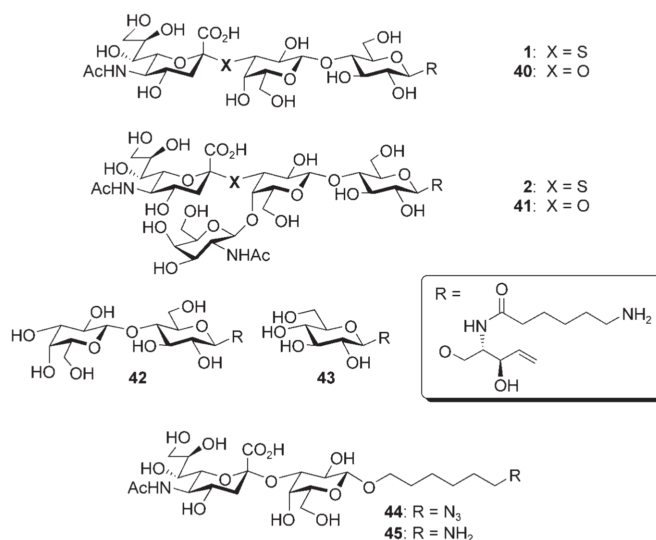



Figure 4. Amine functionalized O- and S-glycosides for conjugation to BSA.

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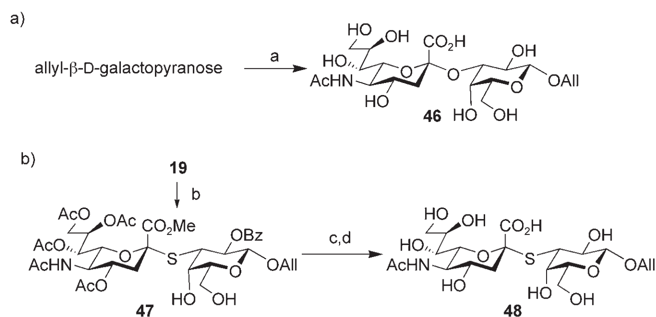
$GM_4$-type disaccharides **46** and **48** (Scheme 7) were designed to serve as soluble ligands for inhibition of immune sera binding to immobilized glycoconjugates and potentially for elaboration to glycoconjugates themselves by functionalization of the terminal olefin. The O-linked $GM_4$ disaccharide **46** was prepared enzymatically from allyl β-D-galactopyranose, while the S-linked analogue **48** was prepared from **19** by hydrolysis of the benzylidene acetal followed by Zemplén transesterification and saponification.




Scheme 7. Synthesis and deprotection of allyl-functionalized disaccharides. a) α(2,3) sialyltransferase, CMP-Neu5Ac, 68%; b) 80% HOAc, 55°C, 85%; c) NaOMe (cat.), MeOH, RT; d) NaOH, MeOH, 40°C, 81% from **19**.

**Immunization**: The synthetic sulfur containing $GM_3$ analogue has been tested for its ability to generate antibodies in mice and we have examined the ability of these antibodies to bind to O-glycosides. Five BALB/c mice were immunized with S-$GM_3$ tetanus toxoid conjugate **36** at days 1, 21, and 28 before collecting sera on day 38. The sera were titrated against an immobilized O-$GM_3$ BSA conjugate (amine **40**, Table 1) to identify cross reactivity with O-glycosides. A strong antigen specific IgG response was observed when immune sera were screened against thiooligosaccharide **1** conjugated via a squarate linker to BSA. In addition, three of five mice gave good Ig titers in the range of $10^3$–$10^4$ against the O-$GM_3$-BSA conjugate as shown in Figure 5. The preponderance of IgG subtype observed is indicative of a class switch and recruitment of T cell help.

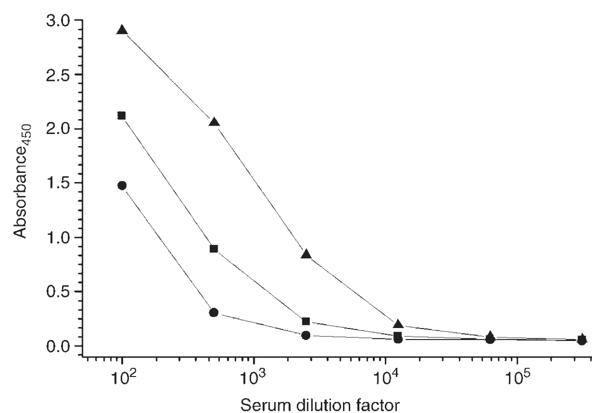



Figure 5. Immunization with S-$GM_3$ tetanus toxoid conjugate **36**. Antisera from five BALB/c mice immunized with **36** were titrated against immobilized O-$GM_3$-BSA. Data are shown for three mice that showed appreciable cross reactivity with the O-glycoside (mouse 1: ■, mouse 2: ●, mouse 3: ▲).

## Conclusion

The chemical and chemoenzymatic synthesis of a number of ganglioside analogues containing S-linked sialic acid residues and a truncated ceramide aglycon have been reported. We have successfully demonstrated that the thiosialoside **1** may serve as an acceptor for bacterial enzymes which catalyze the addition of *N*-acetylgalactosamine (to give $GM_2$ analogue **2**) and *N*-acetylneuraminic acid (to give $GD_3$ analogue **30**), and we have described the conjugation of the O- and S-gangliosides and related molecules to tetanus toxoid and bovine serum albumin. We are currently evaluating the potential of S-linked oligosaccharide–TT conjugates to elicit a carbohydrate specific immune response, and we are analyzing the ability of immune sera to recognize the corresponding O-glycosides. Our initial results indicate that S-glycoside containing tetanus toxoid conjugates are immunogenic and that these antigens stimulate the production of antibodies that recognize O-glycosides.

## Experimental Section

**Chemical synthesis**: All chemical reagents were of analytical grade, used as supplied from Sigma-Aldrich unless indicated. Solvents used in non-hydrolytic reactions were distilled under an inert atmosphere, except *N,N*-dimethylformamide (stored over 3 Å molecular sieves and subjected to reduced pressure for about fifteen minutes (<0.5 mm Hg) prior to use) and pyridine (dried over solid potassium hydroxide and used without further purification). Unless otherwise noted, all reactions were carried out at room temperature and non-hydrolytic reactions were performed under a positive pressure of argon. Solvents were removed between 20 and 40°C (bath temperature) unless indicated otherwise. Molecular sieves were stored in an oven (>170°C) and cooled in vacuo prior to use. Ammonia was distilled once from sodium metal prior to use. Rexyn-101 ($H^+$ form) was used where acidic ion-exchange resin is indicated. Analytical thin layer chromatography (TLC) was conducted on silica gel 60-$F_{254}$ (Merck). Plates were visualized under UV light, and/or by treatment with either acidic cerium ammonium molybdate or 5% ethanolic sulfuric acid, followed by heating.

**Enzymatic synthesis**: Clones of the following recombinant enzymes CMP-Neu5Ac synthetase from *N. meningitidis*,[80] UDP *N*-acetylglucosamine 4-epimerase from *C. jejuni* NCTC 11168,[72] β(1,4) *N*-acetylgalactosaminyl transferase from *C. jejuni* O:36,[73] α(2,3) sialyltransferase[78] and α(2,3/8) sialyltransferase[73] from *Campylobacter jejuni* OH4384 were expressed in *E. coli*, isolated by separation of the pellet and supernatant following cell lysis, diluted or resuspended in the appropriate buffer and used without further purification. The activity of the α(2,3) sialyltransferase and β(1,4) *N*-acetylgalactosaminyl transferases were determined by radiochemical assay by using 8-methoxylcarbonyloctyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside and 8-methoxylcarbonyloctyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside, respectively, as acceptor substrates.[81] CMP-Neu5Ac was prepared as described.[80] Following removal of the protein by centrifugation of the CMP-Neu5Ac

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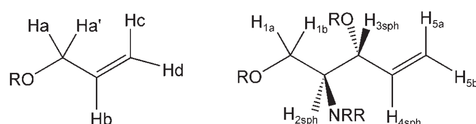
containing solution, the supernatant was lyophilized and the crude solid obtained was used directly in reactions assuming 90% conversion of starting material. A unit of enzyme activity is the amount that catalyzes the formation of 1 μmol of product per minute at 37 °C using the specified acceptor. Alkaline phosphatase was obtained from Roche Diagnostics.

**Chromatography**: Medium pressure chromatography was conducted on silica gel (230–400 mesh, SiliCycle, Montreal) or Iatrobeads 6RS-8060 (Iatron Laboratories Inc., Japan), using a ratio between 50:1 and 125:1 (w/w) of silica gel/crude product at flow rates of 5–10 $mL\,min^{-1}$. Linear gradients were generated using a Q-Grad gradient mixer from Lab Alliance (State College, PA, USA). Reverse phase $C_{18}$ silica gel cartridges ($tC_{18}$ SepPak) were obtained from Waters Corp. (Milford, USA). HPLC purification was conducted on a Waters Delta 600 system by using a UV absorbance detector. Separations were performed on Beckman $C_{18}$-silica semi-preparative or Hamilton PRP-1 reverse phase semi-preparative columns as noted, with combinations of water and methanol (flow rate 1.5–2.5 $mL\,min^{-1}$) as eluents.

**Analytical methods**: $^1H$ and $^{13}C$ NMR spectra were recorded on Varian INOVA 400, 500 and 600 MHz spectrometers. $^1H$ NMR chemical shifts are reported in $\delta$ (ppm) units by using signals from residual solvents as a reference. $^{13}C$ NMR chemical shifts are referenced to internal solvent or to external acetone in the case of $D_2O$. $^1H$ and $^{13}C$ NMR spectra were tentatively assigned with the assistance of COSY, HMQC, HMBC, TOCSY and TROESY spectra as necessary. NMR data for allyl glycosides or compounds containing sphingosine (sph) or ceramide and derivatives as the aglycon were labelled as indicated below.




Electrospray ionization mass spectra were recorded on a Micromass Zabspec TOF mass spectrometer. For high resolution mass determination, spectra were obtained by voltage scan over a narrow range at a resolution of approximately $10^4$. MALDI TOF mass spectra were recorded on a Voyager Elite spectrometer from Applied Biosystems. Optical rotations were determined with a Perkin–Elmer model 241 polarimeter at 22 ± 2 °C, and are reported in units of degrees $mL\,g^{-1}\,dm^{-1}$.

**Immunization**: Eight week old BALB/c mice were immunized by interperitoneal injection of tetanus toxoid conjugate **36** (50 μg) in a 1:1 mixture of PBS and complete Freund's adjuvant. Subsequent injections (50 μg) on days 21 and 28 were made subcutaneously in PBS and incomplete Freund's adjuvant. Mice were bled on day 38 and sera were isolated by centrifugation and then frozen at −20 °C.

**Enzyme linked immunosorbent assay**: O-$GM_3$-squarate-BSA (10 $\mu g\,mL^{-1}$; 100 μL; 4 °C, overnight) was used to coat 96-well ELISA plates (MaxiSorp, Nunc). The plate was washed (Molecular Devices Skan Washer 400) five times with PBST (PBS containing Tween 20, 0.05% v/v). Mouse sera were diluted with PBST containing 0.15 BSA and the solutions were added to the plate and incubated at room temperature for 2 h. The plate was washed with PBST (5×) and goat anti-mouse IgG antibody conjugated to horseradish peroxidase (Kirkegaard & Perry Laboratories; 1:2000 dilution in PBST) was added (100 μL) and incubated for a period of 1 h. The plate was washed five times with PBST before addition of a 1:1 mixture of 3,3′,5,5′-tetramethylbenzidine (0.4 $g\,L^{-1}$) and 0.02% $H_2O_2$ solution (Kirkegaard and Perry Laboratories; 100 μL). After 2 min the reaction was stopped by addition of 1 M phosphoric acid (100 μL). Absorbance was read at 450 nm (Molecular Devices Spectra Max 190 plate reader).

**Compounds**:

**Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-gulopyranoside (12)**: 1,2,3,4,6-Penta-*O*-acetyl-β-D-gulopyranose (20.0 g, 51.2 mmol) was evaporated from toluene (150 mL) and concentrated. The syrup was dissolved in dry dichloromethane (60 mL) and cooled to 0 °C before adding HBr in acetic acid (45% (w/v); 60 mL). After allowing the mixture to warm to RT over 2 h, the solution was evaporated to dryness and then concentrated from toluene (3×60 mL). The yellow residue was then dissolved in dichloromethane (250 mL) and washed with equal volumes of saturated sodium bicarbonate solution followed by water. The organic layers were dried over sodium sulfate, filtered, and concentrated. The syrup obtained was dissolved in allyl alcohol (150 mL) and Drierite (20 g) was added. After stirring the mixture for 30 min, mercury(II) cyanide (7.0 g, 27.7 mmol, 0.54 equiv) and mercury(II) bromide (0.74 g, 2.1 mmol, 0.04 equiv) were added. The reaction mixture was stirred at room temperature under Argon overnight and then concentrated. The residue was suspended in dichloromethane (250 mL), filtered, and washed with 10% potassium bromide solution and water, then dried over sodium sulfate, filtered and concentrated. Column chromatography using a gradient of hexanes and ethyl acetate 3:1 → 5:2 afforded **12** (14.76 g, 38.0 mmol, 74%). $[\alpha]_D = -29$ ($c$ = 1.21, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ = 5.90 (dddd, 1H, $J$=5.0, 6.0, 10.5, 17.2 Hz, H-b), 5.41 (dd, 1H, $J_{3,2}=J_{3,4}$=3.7 Hz, H-3), 5.30 (dddd, 1H, $J$=1.6, 1.6, 1.6, 17.2 Hz, H-c), 5.21 (dddd, 1H, $J$=1.4, 1.4, 1.4, 10.5 Hz, H-d), 5.05 (dd, 1H, $J_{2,1}$=8.0 Hz, H-2), 4.98 (dd, 1H, $J_{4,5}$=1.7 Hz, H-4), 4.81 (d, 1H, H-1), 4.36 (dddd, 1H, $J$=1.5, 1.5, 5.0, 13.1 Hz, H-a), 4.19–4.16 (m, 3H, H-5, H-6a, H-6b), 4.13 (ddd, 1H, $J$=1.4, 1.4, 6.0, 13.1 Hz, H-a′); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 171.4, 170.5, 170.3, 169.8, 134.3, 118.1, 98.0, 70.8, 70.2, 68.8, 68.1, 68.0, 62.2, 20.84, 20.81, 20.79, 20.7; ESI HRMS: $m/z$: calcd for $C_{17}H_{24}O_{10}Na$: 411.1261; found: 411.1262 $[M+Na^+]$; elemental analysis calcd (%) for $C_{17}H_{24}O_{10}$: C 52.57, H 6.23; found: C 52.96, H 6.14.

**Allyl β-D-gulopyranoside (13)**: Tetraacetate **12** (1.488 g, 3.9 mmol) was suspended in anhydrous methanol (10 mL) under a stream of argon. A small piece of sodium metal was added, and the reaction mixture was stirred overnight. The solution was then made neutral by addition of acidic ion exchange resin, and the solids were removed by filtration. A colourless solid was obtained after evaporation of the solvents (0.825 g, 3.7 mmol, 98%). A small portion of this material was further purified for characterization as follows. Silica gel (2 g) was added to a solution of the tetrol (~100 mg) in methanol and the suspension was evaporated to dryness. The solid was applied to the top of a silica gel column, which was then eluted with ethyl acetate/methanol 12:1 to afford pure **13** (87 mg). $[\alpha]_D = -58$ ($c$ = 0.27, MeOH); $^1H$ NMR (600 MHz, $CD_3OD$): $\delta$ = 5.96 (dddd, 1H, $J$=5.3, 6.1, 10.6, 17.2 Hz, H-b), 5.31 (dddd, 1H, $J$=1.8, 1.8, 1.8, 17.2 Hz, H-c), 5.14 (dddd, $J$=1.4, 1.4, 1.8, 10.5 Hz, H-d), 4.63 (d, 1H, $J_{1,2}$=8.2 Hz, H-1), 4.36 (dddd, 1H, $J$=1.6, 1.6, 5.2, 12.9 Hz, H-a), (dddd, 1H, $J$=1.4, 1.4, 6.0, 12.9 Hz, H-a′), 3.94 (dd, 1H, $J_{3,2}=J_{3,4}$=3.6 Hz, H-3), 3.88–3.86 (m, 1H, H-5), 3.74 (dd, 1H, $J_{6a,5}$=6.8, $J_{6a,6b}$=11.5 Hz, H-6a), 3.71–3.67 (m, 2H, H-4, H-6b), 3.63 (dd, 1H, H-2); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 136.0, 117.2, 101.5, 75.0, 73.2, 71.3, 70.8, 69.7, 62.7; ESI HRMS: $m/z$: calcd for $C_9H_{16}O_6Na$: 243.0839; found: 243.0838 $[M+Na^+]$.

**Allyl 4,6-*O*-benzylidene-β-D-gulopyranoside (14)**: Guloside **13** (0.170 g, 0.77 mmol) was dissolved in *N*,*N*-dimethylformamide (3.0 mL) and acetonitrile (3.0 mL). Benzaldehyde dimethyl acetal (0.127 mL, 0.85 mmol, 1.1 equiv) was added, followed by *p*-toluenesulfonic acid monohydrate (6 mg), and the mixture was heated at 50 °C overnight. The reaction mixture was made basic by addition of triethylamine, then concentrated. The residue was dissolved in dichloromethane and acetone, mixed with silica gel (1 g), and concentrated. The residue was applied to a silica gel column and eluted with a linear gradient of hexanes and ethyl acetate (2:1→1:1) to afford **14** (0.175 g, 0.56 mmol, 73%). $[\alpha]_D = -81$ ($c$ = 1.06, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ = 7.53–7.48 (m, 2H, Ar), 7.39–7.31 (m, 3H, Ar), 5.96 (ddd, 1H, $J$=5.3, 6.5, 10.4, 17.0 Hz, H-b), 5.32 (dddd, 1H, $J$=1.6, 1.6, 1.6, 17.1 Hz, H-c), 5.25–5.21 (m, 1H, H-d), 4.75 (d, 1H, $J_{1,2}$=8.2 Hz, H-1), 4.46 (m, 1H, H-a), 4.35 (dd, 1H, $J_{6a,5}$=1.1, $J_{6a,6b}$=12.4 Hz, H-6a), 4.21 (dd, 1H, $J_{3,2}=J_{3,4}$=3.2 Hz, H-3), 4.15–4.06 (m, 3H, H-a′, H-4, H-6b), 3.87 (dd, 1H, H-2), 3.79 (brs, 1H, H-5); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ = 137.7, 134.0, 129.1, 128.2, 126.3, 117.9, 101.3, 99.1, 75.9, 70.0, 69.9, 69.3, 68.9, 66.0; ESI HRMS: $m/z$: calcd for $C_{16}H_{20}O_6Na$: 331.1152; found: 331.1155 $[M+Na^+]$; elemental analysis calcd (%) for $C_{16}H_{20}O_6$: C 62.33, H 6.54; found: C 62.14, H 6.65.

A small amount of the product was acetylated to identify the position of the free hydroxyl groups, yielding allyl 2,3-di-*O*-acetyl-4,6-*O*-benzyl-

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idene-β-D-gulopyranoside: $^1$H NMR (500 MHz, $CDCl_3$): δ = 7.54–7.50 (m, 2H, Ar), 7.40–7.34 (m, 3H, Ar), 5.91 (dddd, 1H, $J$=4.9, 5.8, 10.5, 17.3 Hz, H-b), 5.54 (s, 1H, C*H*Ph), 5.49 (dd, 1H, $J_{3,2}$=$J_{3,4}$=3.4 Hz, H-3), 5.30 (dddd, 1H, $J$=1.7, 1.7, 1.7, 17.3 Hz, H-c), 5.21 (dd, 1H, $J_{2,1}$=8.6 Hz, H-2), 5.21–5.16 (m, 1H, H-d), 4.88 (d, 1H, H-1), 4.40 (dddd, 1H, $J$=1.6, 1.6, 5.0, 13.2 Hz, H-a), 4.35 (dd, 1H, $J_{6a,5}$=1.5, $J_{6a,6b}$=12.6 Hz, H6a), 4.13 (dddd, 1H, $J$=1.4, 1.4, 5.9, 13.2 Hz, H-a′), 4.08 (dd, 1H, $J_{6b,5}$=1.9 Hz, H-6b), 4.01 (dd, 1H, $J_{4,5}$=1.3 Hz, H-4), 3.75 (m, 1H, H-5), 2.15 (s, 3H, $C(O)CH_3$), 2.03 (s, 3H, $C(O)CH_3$).

When the initial reaction was carried out under identical conditions using *N*,*N*-dimethylformamide or acetonitrile as the lone solvent, slightly lower yields were realized, and a second isomer (~5%), identified as allyl (*R*)-4,6-*O*-benzylidene-β-D-gulopyranoside could be recrystallized (ethyl acetate/hexanes) after chromatography as described above. $[\alpha]_D$ = −81 ($c$ = 0.89, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): δ = 7.50–7.46 (m, 2H, Ar), 7.44–7.39 (m, 2H, Ar), 7.37–7.34 (m, 1H, Ar), 6.14 (s, 1H, C*H*Ph), 5.97 (dddd, 1H, $J$=5.6, 6.4, 10.4, 16.9 Hz, H-b), 5.33 (dddd, 1H, $J$=1.6, 1.6, 1.6, 17.1 Hz, H-c), 5.24 (dddd, 1H, $J$=10.3, 1.3, 1.3, 1.3 Hz, H-d), 4.71 (d, 1H, $J_{1,2}$=7.7 Hz, H-1), 4.46 (m, 1H, H-a), 4.26 (dd, 1H, $J_{3,2}$=$J_{3,4}$=2.5 Hz, H-3), 4.12 (m, 1H, H-a′), 4.06–4.01 (m, 3H, H-4, H-$6_a$, H-$6_b$), 3.95 (dd, 1H, H-2), 3.78 (m, 1H, H-5), 2.55 (brs, 1H, O*H*), 2.49 (brs, 1H, O*H*); $^{13}$C NMR (125 MHz, $CDCl_3$): δ = 137.7, 134.0, 129.1, 128.2, 126.3, 117.9, 101.3, 99.1, 75.9, 70.0, 69.9, 69.3, 68.9, 66.0; ESI HRMS: $m/z$: calcd for $C_{16}H_{20}O_6Na$: 331.1152; found: 331.1154 [$M$+$Na^+$]; elemental analysis calcd (%) for $C_{16}H_{20}O_6$: C 62.33, H 6.54; found: C 62.09, H 6.51.

A small amount of the product was acetylated to identify the position of the free hydroxyl groups, yielding allyl 2,3-di-*O*-acetyl-(*R*)-4,6-*O*-benzylidene-β-D-gulopyranoside: $^1$H NMR (600 MHz, $CDCl_3$): δ = 7.47–7.44 (m, 2H, Ar), 7.39–7.32 (m, 3H, Ar), 5.99 (s, 1H, C*H*Ph), 5.95–5.87 (m, 2H, H-3, H-b), 5.33 (dddd, 1H, $J$=1.6, 1.6, 1.6, 17.4 Hz, H-c), 5.30 (dd, 1H, $J_{2,3}$=3.4, $J_{2,1}$=4.4 Hz, H-2), 5.24 (dddd, 1H, $J$=1.4, 1.4, 1.4, 10.5 Hz, H-d), 4.82 (d, 1H, $J_{1,2}$=4.5 Hz, H-1), 4.34–4.24 (m, 3H, H-4, $OCH_2CHCH_2$, H-6a), 4.19 (m, 1H, H-5), 4.12–4.04 (m, 2H, H-6b, $OCH_2CHCH_2$), 2.12, 2.08 (2s, 6H, 2×$C(O)CH_3$).

**Allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-gulopyranoside (15)**: Diol **14** (4.00 g, 13.0 mmol) was dissolved in a mixture of dry dichloromethane and pyridine (4:1; 60 mL), and the flask was immersed in an ice bath. Benzoyl chloride (1.66 mL, 14.3 mmol, 1.1 equiv) was added, and after 40 min the reaction mixture was quenched by addition of methanol (5 mL). After stirring for 20 min at room temperature the solution was concentrated. The residue was dissolved in dichloromethane (100 mL), washed successively with 1 M HCl, saturated sodium bicarbonate, and water, then dried over sodium sulfate, filtered and concentrated. Column chromatography in hexanes/ethyl acetate 2:1 yielded **15** (5.01 g, 12.1 mmol, 94%). $[\alpha]_D$ = −40 ($c$ = 0.76, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$): δ = 8.07–8.04 (m, 2H, Ar), 7.62–7.58 (m, 1H, Ar), 7.57–7.54 (m, 2H, Ar), 7.49–7.45 (m, 2H, Ar), 7.41–7.35 (m, 3H, Ar), 5.85 (dddd, 1H, $J$=5.0, 6.1, 10.6, 17 Hz, H-b), 5.59 (s, 1H, C*H*Ph), 5.41 (dd, 1H, $J_{2,1}$=8.5, $J_{2,3}$=3.3 Hz, H-2), 5.26 (dddd, 1H, $J$=1.6, 1.6, 1.6, 17.2 Hz, H-c), 5.13 (dddd, 1H, $J$=1.4, 1.4, 1.4, 10.6 Hz, H-d), 5.11 (d, 1H, H-1), 4.44–4.36 (m, 2H, H-a, H-6a), 4.19–4.10 (m, 2H, H-a′, H-6b), 3.89 (m, 1H, H-5); $^{13}$C NMR (125 MHz, $CDCl_3$): δ = 165.1, 137.6, 134.1, 133.3, 129.82, 129.77, 129.1, 128.5, 128.2, 126.4, 117.1, 101.3, 96.9, 76.3, 71.3, 69.5, 69.28, 69.27, 65.7; ESI HRMS: $m/z$: calcd for $C_{23}H_{24}O_7Na$: 435.1414; found: 435.1414 [$M$+$Na^+$]; elemental analysis calcd (%) for $C_{23}H_{24}O_7$: C 66.98, H 5.87; found: C 67.06, H 5.79.

A second, less polar compound allyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-gulopyranoside (**16**) was also isolated (0.064 g, 0.12 mmol, 1%). $[\alpha]_D$ = −88 ($c$ = 0.35, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): δ = 8.08–8.05 (m, 2H, Ar), 7.92–7.87 (m, 2H, Ar), 7.67–7.62 (m, 1H, Ar), 7.60–7.56 (m, 2H, Ar), 7.53–7.47 (m, 3H, Ar), 7.43–7.36 (m, 3H, Ar), 7.35–7.29 (m, 2H, Ar), 5.93–5.83 (m, 2H, H-3, H-b), 5.63 (dd, 1H, $J_{2,1}$=8.4, $J_{2,3}$=3.5 Hz, H-2), 5.62 (s, 1H, C*H*Ph), 5.29 (dddd, 1H, $J$=1.7, 1.7, 1.7, 17.2 Hz, H-c), 5.23 (d, 1H, H-1), 5.14 (dddd, 1H, $J$=1.4, 1.4, 1.4, 10.6 Hz, H-d), 4.49–4.39 (m, 2H, H-a, H-6a), 4.26–4.19 (m, 2H, H-4, H-a′), 4.14 (m, 1H, H-6b), 3.93 (m, 1H, H-5); $^{13}$C NMR (125 MHz, $CDCl_3$): δ = 164.93, 164.91, 137.3, 134.0, 133.6, 133.0, 129.8, 129.71, 129.69, 129.4, 129.2, 128.7, 128.25, 128.22, 126.4, 117.0, 101.3, 97.7, 74.3, 70.5, 69.4, 69.1, 68.6, 66.3; ESI HRMS: $m/z$: calcd for $C_{30}H_{28}O_8Na$: 539.1676; found: 539.1679; elemental analysis calcd (%) for $C_{30}H_{28}O_8$: C 69.76, H 5.46; found: C 69.66, H 5.65.

**Allyl 3-thioacetyl-2-*O*-benzoyl-4,6-*O*-benzylidene-3*S*-β-D-galactopyranoside (17)**: Alcohol **15** (2.20 g, 5.3 mmol) was dissolved in dichloromethane (40 mL) containing pyridine (2.10 mL, 27 mmol, 5 equiv). The solution was cooled to −20°C and trifluoromethanesulfonic anhydride (1.20 mL, 7.1 mmol, 1.34 equiv) was added over a period of one minute. The mixture was allowed to warm to −5°C over 1.5 h, and was then stirred at room temperature for a further 1.5 h. After diluting with dichloromethane (40 mL), the reaction mixture was washed with an equal volume of water, dried over sodium sulfate, filtered, and concentrated. The residue was dissolved in *N*,*N*-dimethylformamide (50 mL), potassium thioacetate (1.30 g, 11.4 mmol, 2 equiv) was added, and mixture was immersed in an oil bath at 50°C. After stirring overnight, the black solution was concentrated. The syrup was dissolved in dichloromethane (50 mL) and washed with an equal volume of water, dried over sodium sulfate, filtered and concentrated. The yellow residue was chromatographed on a silica gel column (3.5 cm×30 cm) capped by a 2 cm deep plug of decolourising carbon by using hexanes/ethyl acetate 2:1 as eluent to yield **17** (2.36 g, 5.0 mmol, 94%). $[\alpha]_D$ = 156 ($c$ = 0.48, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$): δ = 8.01–7.99 (m, 2H, Ar), 7.58–7.52 (m, 3H, Ar), 7.46–7.42 (m, 2H, Ar), 7.41–7.36 (m, 3H, Ar), 5.77 (dddd, 1H, $J$=4.8, 6.2, 10.8, 17.2 Hz, H-b), 5.56 (s, 1H, C*H*Ph), 5.49 (dd, 1H, $J_{2,1}$=7.7, $J_{2,3}$=11.5 Hz, H-2), 5.20 (dddd, 1H, $J$=1.7, 1.7, 1.7, 17.2 Hz, H-c), 5.09 (dddd, 1H, $J$=1.4, 1.4, 1.4, 10.8 Hz, H-d), 4.78 (d, 1H, H-1), 4.39–4.35 (m, 2H, H-6a, H-a), 4.25 (dd, 1H, $J_{3,4}$=3.3 Hz, H-3), 4.17–4.07 (m, 3H, H-4, H-6b, H-a′), 3.70 (m, 1H, H-5), 2.21 (s, 3H, $C(O)CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): δ = 194.9, 165.2, 137.4, 133.8, 133.0, 129.8, 129.7, 129.0, 128.3, 128.2, 126.3, 117.2, 101.3, 101.1, 75.9, 69.3, 69.04, 69.01, 68.5, 47.0, 30.5; ESI HRMS: $m/z$: calcd for $C_{25}H_{26}O_7SNa$: 493.129695; found: 493.129366 [$M$+$Na^+$]; elemental analysis calcd (%) for $C_{25}H_{26}O_7S$: C 63.81, H 5.57; found: C 63.95, H 5.51.

**Allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (18)**: Thioester **17** (0.320 g, 0.68 mmol) was dissolved in *N*,*N*-dimethylformamide (5.0 mL), and argon was bubbled through the solution for 20 min before hydrazine acetate (0.113 g, 1.23 mmol, 1.7 equiv) was added. The mixture was stirred for 2 h, diluted with ethyl acetate (50 mL), washed with water (2×40 mL), and the organic layers dried over sodium sulfate, filtered and concentrated to afford **18** (0.286 g, 0.67 mmol, 98%). $^1$H NMR (400 MHz, $CDCl_3$): δ = 8.09–8.05 (m, 2H, Ar), 7.59–7.55 (m, 3H, Ar), 7.49–7.37 (m, 5H, Ar), 5.79 (dddd, 1H, $J$=4.8, 6.2, 11.0, 17.3 Hz, H-b), 5.61 (s, 1H, C*H*Ph), 5.37 (dd, 1H, $J_{2,1}$=7.8, $J_{2,3}$=11.2 Hz, H-2), 5.21 (dddd, 1H, $J$=1.7, 1.7, 1.7, 17.3 Hz, H-c), 5.09 (dddd, 1H, $J$=1.4, 1.4, 1.4, 10.5 Hz, H-d), 4.66 (d, 1H, H-1), 4.38 (dd, 1H, $J_{6a,6b}$=12.4, $J_{6a,5}$=1.5 Hz, H-6a), 4.39–4.33 (m, 1H, H-a), 4.17–4.09 (m, 3H, H-4, H-a′, H-6b), 3.61 (m, 1H, H-5), 3.14 (ddd, 1H, $J_{3,4}$=3.2, $J_{3,2}$=11.0, $J_{3,SH}$=11.0 Hz, H-3), 2.24 (d, 1H, S*H*); $^{13}$C NMR (125 MHz, $CDCl_3$): δ = 165.4, 137.5, 133.9, 133.0, 130.1, 129.8, 129.1, 128.3, 128.2, 128.3, 117.2, 101.5, 101.0, 76.6, 72.5, 69.2, 69.03, 68.96, 43.8; ESI HRMS: $m/z$: calcd for $C_{23}H_{24}O_6SNa$: 451.1191; found: 451.1191 [$M$+$Na^+$].

The title compound was slowly converted to allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside disulfide in solution: $^1$H NMR (500 MHz, $CDCl_3$): δ = 8.09–8.06 (m, 2H, Ar), 7.60–7.53 (m, 3H, Ar), 7.47–7.43 (m, 2H, Ar), 7.40–7.34 (m, 3H, Ar), 5.78 (dddd, 1H, $J$=4.8, 6.1, 10.6, 17.2 Hz, H-b), 5.60 (s, 1H, C*H*Ph), 5.59 (dd, 1H, $J_{2,1}$=7.8, $J_{2,3}$=11.2 Hz, H-2), 5.20 (dddd, 1H, $J$=1.6, 1.6, 1.6, 17.2 Hz, H-c), 5.08 (dddd, 1H, $J$=1.6, 1.6, 1.6, 10.6 Hz, H-d), 4.71 (d, 1H, H-1), 4.39–4.34 (m, 2H, H-6a, H-a), 4.33 (dd, 1H, $J$=0.9, 3.2 Hz, H-4), 4.16–4.09 (m, 2H, H-6b, H-a′), 3.56 (brs, 1H, H-5), 2.99 (dd, 1H, H-3); $^{13}$C NMR (125 MHz, $CDCl_3$): δ = 165.2, 137.6, 133.9, 133.0, 130.1, 129.8, 128.9, 128.4, 128.1, 126.3, 117.1, 101.4, 101.2, 78.4, 69.9, 69.2, 69.1, 68.9, 49.3; ESI HRMS: $m/z$: calcd for $C_{46}H_{46}O_{12}S_2Na$: 877.2328; found: 877.2327 [$M$+$Na^+$]; elemental analysis calcd (%) for $C_{46}H_{46}O_{12}S_2$: C 62.93, H 5.28; found: C 63.18, H 5.40.

**Allyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-2-*O*-benzoyl-(*S*)-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (19)**: Thiol **18** (0.613 g,


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1.43 mmol) was dissolved in *N*,*N*-dimethylformamide (15 mL) and argon was bubbled through for one hour prior to cooling the solution to 0 °C. Sodium hydride (dispersion in mineral oil; 60 % by mass; 0.074 g, 1.85 mmol, 1.3 equiv) was added in portions and stirring was continued until the evolution of gas had ceased. A solution of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate[82] (1.30 g, 2.55 mmol, 1.8 equiv) and 1,4,10-trioxa-7,13-diazacyclopentadecane (0.062 g, 0.29 mmol, 0.2 equiv) in *N*,*N*-dimethylformamide (5 mL) was added via canula and the mixture was stirred for 40 min, after which time the mixture was neutralized with acetic acid. The solution was diluted with ethyl acetate (150 mL) and washed with an equal volume of water, then dried over sodium sulfate, filtered and concentrated. Column chromatography on silica gel with a linear gradient of hexanes/ethyl acetate/acetone 4:3:1→4:3:2) afforded **19** (1.220 g, 1.35 mmol, 95 %). $[\alpha]_D$ = 50 ($c$ = 0.27, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$): δ = 8.16–8.12 (m, 2H, Ar), 7.58–7.52 (m, 3H, Ar), 7.49–7.45 (m, 2H, Ar), 7.39–7.31 (m, 3H, Ar), 5.79 (dddd, 1H, $J$=5.1, 5.9, 10.5, 17.2 Hz, H-b), 5.63 (ddd, 1H, $J$=2.5, 5.6, $J_{8',7'}$=10.0 Hz, H-8′), 5.48 (s, 1H, C*H*Ph), 5.30 (dd, 1H, $J_{2,1}$=7.7, $J_{2,3}$=11.8 Hz, H-2), 5.23 (dd, 1H, $J_{7',6'}$=2.2 Hz, H-7′), 5.18 (dddd, 1H, $J$=1.7, 1.7, 1.7, 17.2 Hz, H-c), 5.06–5.01 (m, 2H, H-1, H-d), 4.83 (ddd, 1H, $J_{4',3'eq}$=4.6, $J_{4',5'}$=10.4, $J_{4',3'ax}$=11.6 Hz, H-4′), 4.38–4.31 (m, 3H, H-9a′, H-6a, H-a), 4.15 (dddd, 1H, $J$=1.4, 1.4, 6.0, 13.1 Hz, H-a′), 4.12–4.05 (m, 2H, H-9b′, H-6b), 3.93–3.85 (m, 2H, H-5′, H-3), 3.80–3.73 (m, 3H, H-5, H-4, H-6′), 3.77 (s, 3H, $CO_2CH_3$), 2.58 (dd, 1H, $J_{3eq,3ax}$=12.9 Hz, H-3′$_{eq}$), 1.91 (dd, 1H, H-3′$_{ax}$), 2.21, 2.07, 1.97, 1.84, 1.70 (5s, 5×3H, 5×C(O)C*H*$_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 170.9, 170.7, 170.18, 170.14, 170.12, 169.8, 165.9, 137.6, 134.1, 132.8, 130.6, 130.1, 128.9, 128.2, 128.1, 126.3, 116.9, 101.1, 100.9, 81.0, 76.3, 73.6, 69.6, 69.4, 69.2, 69.1, 67.9, 67.5, 66.9, 62.4, 53.0, 49.3, 45.6, 37.4, 23.2, 21.5, 20.8, 20.6; ESI HRMS: *m*/*z*: calcd for $C_{43}H_{51}NO_{18}SNa$: 924.2719; found: 924.2720 [$M$+$Na^+$]; elemental analysis calcd (%) for $C_{43}H_{51}NO_{18}S$: C 57.26, H 5.70, N 1.55; found: C 56.87, H 5.56, N 1.40.

**2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-3-*O*-benzoyl-pent-4-ene-1,3-diol (20)**: A suspension of the imidate **8** (0.301 g, 0.61 mmol, 1.28 equiv), (2*S*,3*R*)-2-azido-3-*O*-benzoyl-pent-4-ene-1,3-diol (**9**) (0.117 g, 0.47 mmol, 1.00 equiv) and powdered 4 Å molecular sieves (100 mg) were combined in freshly distilled dichloromethane (10.0 mL) and stirred for 30 min under an argon atmosphere at room temperature. The mixture was then cooled to −50 °C and trimethylsilyl trifluoromethanesulfonate (20 μL in 0.5 mL $CH_2Cl_2$) was added. The reaction mixture was allowed to warm to room temperature and stirring was continued for 6 h before triethylamine was added to neutralize the mixture. After filtration and concentration, the residue was chromatographed on silica gel by using hexanes/ethyl acetate 4:1 as eluent to afford **20** (0.248 g, 4.3 mmol, 90 %). $[\alpha]_D$ = −59 ($c$ = 1.52, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$): δ = 8.09–8.06 (m, 2H, Ar), 7.62–7.57 (m, 1H, Ar), 7.50–7.41 (m, Ar, 4H), 7.37–7.33 (m, 3H, Ar), 5.96 (ddd, 1H, $J$=7.1, 10.6, 17.2 Hz, H-4$_{sph}$), 5.67–5.63 (m, 1H, H-3$_{sph}$), 5.52–5.46 (m, 2H, C*H*Ph, H-5$_{sph}$), 5.45–5.40 (m, 1H, H-5$_{sph}$), 5.31 (dd, 1H, $J_{3,2}$=$J_{3,4}$=9.4 Hz, H-3), 5.04 (dd, 1H, $J_{2,1}$=7.8 Hz, H-2), 4.29 (dd, 1H, $J_{6a,6b}$=10.5, $J_{6a,5}$=5.0 Hz, H-6a), 4.01–3.90 (m, 2H, C-2$_{sph}$, C-1$_{sph}$), 3.75–3.64 (m, 3H, H-4, H-6b, C-1$_{sph}$), 3.53 (ddd, 1H, $J$=5.0, 9.9, 9.9 Hz, H-5), 2.10 (s, 3H, C(O)C*H*$_3$) 2.05 (s, 3H, C(O)C*H*$_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 170.1, 169.5, 165.0, 136.7, 133.4, 131.4, 129.8, 129.7, 129.2, 128.5, 128.2, 126.1, 120.8, 101.5, 101.0, 78.2, 74.4, 72.1, 71.8, 68.4, 68.2, 66.5, 63.2, 20.8, 20.7; ESI HRMS: *m*/*z*: calcd for $C_{29}H_{31}N_3O_{10}Na$: 604.1902; found: 604.1907 [$M$+$Na^+$]; elemental analysis calcd (%) for $C_{29}H_{31}N_3O_{10}$: C 59.89, H 5.37, N 7.23; found: C 59.61, H 5.20, N 7.09.

A side product, (2*S*,3*R*)-1-*O*-acetyl-2-azido-3-*O*-benzoyl-pent-4-ene-1,3-diol (**21**) was isolated (0.007 g, 0.001 mmol, 4 %). $^1H$ NMR (400 MHz, $CDCl_3$): δ = 8.10–8.06 (m, 2H, Ar), 7.63–7.57 (m, 1H, Ar), 7.51–7.45 (m, 2H, Ar), 6.01–5.91 (m, 1H, H-4), 5.69–5.64 (m, 1H, H-3), 5.54–5.47 (m, 1H, H-5a), 5.45–5.41 (m, 1H, H-5b), 4.28 (dd, 1H, $J_{1a,2}$=4.6, $J_{1a,1b}$=11.6 Hz, H-1a), 4.19 (dd, 1H, $J_{1b,2}$=7.8 Hz, H-1b), 4.02 (ddd, 1H, $J$=4.5, 4.5, 7.8 Hz, H-2), 2.12 (s, 3H, C(O)C*H*$_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 170.4, 165.1, 133.5, 131.2, 129.8, 129.4, 128.5, 120.8, 74.3, 62.9, 62.6, 20.7; ESI HRMS: *m*/*z*: calcd for $C_{14}H_{15}N_3O_4Na$: 312.09548; found: 312.09526 [$M$+$Na^+$].

**2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-3-*O*-benzyl-pent-4-ene-1,3-diol (22)**: Diacetate **20** (1.053 g, 1.81 mmol) was dissolved in dry methanol (30 mL) and a small piece of sodium was added. After 3 h, the mixture was neutralized with acidic cation exchange resin, filtered, and concentrated. The residue was dissolved in anhydrous *N*,*N*-dimethylformamide (25 mL) and cooled to 0 °C. Sodium hydride (0.434 g of a 60 % dispersion in oil; 10.9 mmol, 6 equiv) was added in portions, followed by benzyl bromide (1.3 mL, 10.9 mmol, 6 equiv). After 4 h methanol was added followed by acidic cation exchange resin. The neutral solution was filtered and concentrated, then redissolved in dichloromethane (100 mL) and washed with water (2× 60 mL), dried and concentrated. The residue was chromatographed on silica gel with hexanes/ethyl acetate 10:1 as eluent to give **22** (1.136 g, 1.71 mmol, 94 %). $[\alpha]_D$ = −43 ($c$ = 1.21, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$): δ = 7.51–7.48 (m, 2H, Ar), 7.41–7.25 (m, 18H, Ar), 5.84 (ddd, 1H, $J$=7.9, 10.4, 18.3 Hz, H-4$_{sph}$), 5.57 (s, 1H, C*H*Ph), 5.44–5.41 (m, 1H, H-5a$_{sph}$), 5.38–5.33 (m, 1H, H-5b$_{sph}$), 4.92 (d, 1H, $J$=11.4 Hz, C*H*$_2$Ph), 4.84 (d, 1H, $J$=11.0 Hz, C*H*$_2$Ph), 4.80 (d, 1H, $J$=11.4 Hz, C*H*$_2$Ph), 4.78 (d, 1H, $J$=11.0 Hz, C*H*$_2$Ph), 4.62 (d, 1H, $J$=11.9 Hz, C*H*$_2$Ph), 4.51 (d, 1H, $J_{1,2}$=7.6 Hz, H-1), 4.38 (d, 1H, $J$=11.4 Hz, C*H*$_2$Ph), 4.35 (dd, 1H, $J$=10.4, 5.0 Hz, H-6a), 4.00 (dd, 1H, $J$=6.7, 10.0 Hz, H-1a$_{sph}$), 3.95 (dd, 1H, $J$=5.3, 7.9 Hz, H-3$_{sph}$), 3.81–3.67 (m, H-6b, H-1b$_{sph}$, H-3, H-4, H-2$_{sph}$), 3.48 (dd, 1H, $J_{2,3}$=8.3 Hz, H-2), 3.44–3.37 (m, 1H, H-5); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 138.5, 138.3, 137.8, 137.3, 134.3, 128.9, 128.4, 128.34, 128.28, 128.23, 127.99, 127.96, 127.72, 127.68, 127.63, 127.61, 126.0, 120.8, 103.9, 101.2, 82.1, 81.5, 80.9, 79.8, 75.4, 75.1, 70.5, 68.8, 68.7, 66.1, 64.2; ESI HRMS: *m*/*z*: calcd for $C_{39}H_{41}N_3O_7Na$: 686.2837; found: 686.2838 [$M$+$Na^+$]; elemental analysis calcd (%) for $C_{39}H_{41}N_3O_7$: C 70.57, H 6.23, N 6.33; found: C 70.94, H 6.40, N 6.07.

**2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(*N*-carboxybenzyl-6-aminohexanamido)-3-*O*-benzyl-pent-4-ene-1,3-diol (24)**: Azide **22** (0.098 g, 0.15 mmol) was dissolved in a mixture of pyridine and water (20:1; 5 mL) and triphenylphosphine (0.078 g, 0.29 mmol, 2.0 equiv) was added. The solution was heated at 50 °C for 4.5 h and then cooled to room temperature. 6-(Benzyloxycarbonylamino)hexanoic acid succinimidyl ester (0.107 g, 0.29 mmol, 2.0 equiv) was added to the flask, and after heating overnight at 50 °C the mixture was concentrated. Chromatography on silica gel using a linear gradient of methanol in dichloromethane (5.0→6.2 %) as eluent afforded **24** (0.112 g, 0.13 mmol, 85 %). $[\alpha]_D$ = −25.09 ($c$ = 0.31, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.51–7.48 (m, 2H, Ar), 7.41–7.25 (m, 23H, Ar), 5.75 (ddd, 1H, $J$=7.9, 10.3, 17.3 Hz, H-4$_{sph}$), 5.71 (d, 1H, $J$=9.4 Hz, N*H*C(O)$CH_2$), 5.58 (s, 1H, C*H*Ph), 5.31–5.27 (m, 1H, H-5a$_{sph}$), 5.23–5.18 (m, 1H, H-5b$_{sph}$), 5.09 (s, 2H, C*H*$_2$Ph), 4.94 (d, 1H, $J$=11.5 Hz, C*H*$_2$Ph), 4.83 (d, 1H, $J$=11.1 Hz, C*H*$_2$Ph), 4.80 (d, 1H, $J$=11.5 Hz, C*H*$_2$Ph), 4.76 (br t, 1H, N*H*$CO_2$Bn), 4.69 (d, 1H, $J$=11.1 Hz, C*H*$_2$Ph), 4.62 (d, 1H, $J$=11.9 Hz, C*H*$_2$Ph), 4.45 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 4.35 (d, 1H, $J$=11.9 Hz, C*H*$_2$Ph), 4.34 (dd, 1H, $J_{6a,5}$=5.0, $J_{6a,6b}$=10.4 Hz, H-6a), 4.29 (dd, 1Hx, $J_{1a,2}$=3.9, $J_{1a,1b}$=10.5 Hz, H-6a), 4.23 (m, 1H, H-2$_{sph}$), 3.85 (dd, 1H, $J_{3,2}$=$J_{3,4}$=7.6 Hz, H-3$_{sph}$), 3.80–3.75 (m, 2H, H-3, H-6b), 3.70 (dd, 1H, $J_{4,3}$=$J_{4,5}$=9.4 Hz, H-4), 3.61 (dd, 1H, $J_{1,2}$=3.7 Hz, H-1b$_{sph}$), 3.44 (dd, 1H, $J_{2,3}$=8.7 Hz, H-2), 3.39 (ddd, 1H, $J_{5,6b}$=9.6 Hz, H-5), 3.16–3.11 (m, 2H, C*H*$_2$NH$CO_2$Bn), 1.89–1.75 (m, 2H, C(O)C*H*$_2$), 1.49–1.38 (m, 4H, 2×C*H*$_2$), 1.22–1.15 (m, 2H, C*H*$_2$); $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 172.3, 156.4, 138.3, 138.1 (2C), 137.3, 136.6, 135.7, 129.0, 128.51, 128.46, 128.35, 128.33, 128.25, 128.11, 128.06, 128.04, 127.87, 127.84, 127.80, 127.70, 127.66, 126.0, 119.6, 104.4, 101.2, 82.0, 81.7, 81.1, 79.5, 75.4, 75.0, 70.6, 68.8, 68.7, 66.6, 66.1, 51.7, 40.9, 36.2, 29.6, 26.2, 25.0; ESI HRMS: *m*/*z*: calcd for $C_{53}H_{60}N_2O_{10}Na$: 907.4146; found: 907.4145 [$M$+$Na^+$]; elemental analysis calcd (%) for $C_{53}H_{60}N_2O_{10}$: C 71.92, H 6.83, N 3.17; found: C 71.73, H 6.71, N 2.96.

**2,3,6-Tri-*O*-benzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-3-*O*-benzyl-pent-4-ene-1,3-diol (5)**: Azide **22** (0.720 g, 1.08 mmol) was concentrated twice from toluene and the residue was dissolved in tetrahydrofuran (20 mL) containing powdered 3 Å molecular sieves. After stirring for 20 min, sodium cyanoborohydride (0.681 g, 10.84 mmol, 10 equiv) was added, followed by a crystal of methyl orange. A saturated solution of hydrochloric acid in diethyl ether was added to the mixture, dropwise, until a pink colour persisted. After 90 min, an additional solution of the acid (3 mL) was added. After a total of 2.5 h, the reaction mixture was

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filtered into a separatory funnel containing a 1:1 mixture of dichloromethane and water (150 mL). The organic layer was removed, dried over sodium sulfate and concentrated. Chromatography of the residue, by using hexanes/ethyl acetate 5:1 as eluent afforded **5** (0.685 g, 1.03 mmol, 95 %). $[\alpha]_D = -34.30$ ($c = 0.52$, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$): $\delta = 7.36$–7.24 (m, 24H, Ar), 5.83 (m, 1H, $J=8.0$, 10.4, 17.4 Hz, $OCH_2CHCH_2$), 5.42–5.39 (m, 1H, $OCH_2CHCH_2$), 5.35–5.31 (m, 1H, $OCH_2CHCH_2$), 4.92 (d, 1H, $J=11.6$ Hz, $OCH_2Ph$), 4.87 (d, 1H, $J=11.3$ Hz, $OCH_2Ph$), 4.74 (d, 1H, $J=11.6$ Hz, $OCH_2Ph$), 4.71 (d, 1H, $J=11.2$ Hz, $OCH_2Ph$), 4.62 (d, 1H, $J=11.8$ Hz, $OCH_2Ph$), 4.59 (d, 1H, $J=12.0$ Hz, $OCH_2Ph$), 4.55 (d, 1H, $J=12.0$ Hz, $OCH_2Ph$), 4.41 (d, 1H, $J_{1,2}=7.3$ Hz, H-1), 4.38 (d, 1H, $J=11.8$ Hz, $OCH_2Ph$), 4.02 (dd, 1H, $J_{6a,5}=6.7$, $J_{6a,6b}=10.2$ Hz, H-6a), 3.96 (dd, 1H, $J=5.3$, 7.9 Hz, H-$3_{sph}$), 3.78–3.60 (m, 5H, H-4, H-5, H-6b, H-$2_{sph}$, H-$1a_{sph}$), 3.49–3.40 (m, 3H, H-$1b_{sph}$, H-2, H-3); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta = 138.6$, 138.4, 137.87 (2C), 134.3, 128.5, 128.42, 128.38, 128.37, 128.0, 127.9, 127.8, 127.7, 127.68, 127.67, 127.65, 127.62, 120.7, 103.5, 84.0, 81.7, 79.8, 75.3, 74.8, 74.1, 73.7, 71.6, 70.4, 70.2, 68.4, 64.2; ESI HRMS: $m/z$: calcd for $C_{34}H_{36}O_6Na$: 688.2993; found: 688.3001 $[M+Na^+]$; elemental analysis calcd (%) for $C_{34}H_{36}O_6$: C 70.36, H 6.51, N 6.31; found: C 70.46, H 6.68, N 6.06.

**2,3,6-Tri-*O*-benzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(*N*-carboxybenzyl-6-aminohexanamido)-3-*O*-benzyl-pent-4-ene-1,3-diol (23)**: Azido alcohol **5** (0.201 g, 0.30 mmol) was taken up in pyridine/water 9:1 (10 mL) and triphenylphosphine (160 mg, 0.61 mmol, 2 equiv) was added. The mixture was stirred at 45 °C for 3 h and then cooled to room temperature. 6-(Benzyloxycarbonylamino)-hexanoic acid succinimidyl ester (0.383 g, 3.5 equiv) was added and the reaction mixture was stirred at 45 °C for a further 2.5 h, then concentrated. The residue was chromatographed on silica gel by using a linear gradient of dichloromethane (15:1 → 12:1) as eluent to yield **23** (0.211 g, 0.24 mmol, 79 %). $[\alpha]_D = -17.30$ ($c = 0.23$, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$): $\delta = 7.39$–7.31 (m, 25H, Ar), 5.79 (d, 1H, $NHC(O)CH_2$), 5.74 (ddd, 1H, $J=7.9$, 10.5, 17.5 Hz, H-$4_{sph}$), 5.28 (brd, 1H, $J=10.6$ Hz, H-$5a_{sph}$), 5.20 (brd, 1H, $J=17.1$ Hz, H-$5b_{sph}$), 5.09 (s, 2H, $CH_2Ph$), 4.82 (d, 1H, $J=11.9$ Hz, $CH_2Ph$), 4.80 (d, 1H, $J=11.9$ Hz, $CH_2Ph$), 4.79 (brs, 1H, $CH_2NH$), 4.76 (s, 2H, $CH_2Ph$), 4.61 (d, 1H, $J=12.0$ Hz, $CH_2Ph$), 4.58 (d, 1H, $J=11.9$ Hz, $CH_2Ph$), 4.54 (d, 1H, $J=11.9$ Hz, $CH_2Ph$), 4.36 (d, 1H, $J_{1,2}=7.4$ Hz, H-1), 4.35 (d, 1H, $J=11.9$ Hz, $CH_2Ph$), 4.25 (dd, 1H, $J=4.5$, 10.4 Hz, H-$1a_{sph}$), 4.27–4.18 (m, 1H, H-$2_{sph}$), 3.88 (dd, 1H, $J_{3,4}=7.3$ Hz, H-$3_{sph}$), 3.75 (dd, 1H, $J_{6a,5}=4.2$, $J_{6a,6b}=10.3$ Hz, H-6a), 3.71 (dd, 1H, $J_{6b,5}=5.1$ Hz, H-6b), 3.67–3.62 (m, 2H, H-$1b_{sph}$, H-4), 3.48 (dd, 1H, $J_{3,2}=J_{3,4}=9.0$ Hz, H-3), 3.42 (ddd, 1H, $J_{5,4}=9.4$ Hz, H-5), 3.38 (dd, 1H, H-2), 3.16–3.09 (m, 2H, $CH_2NH_2$), 1.91–1.79 (m, 2H), 1.51–1.37 (m, 4H), 1.22–1.17 (m, 2H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta = 172.4$, 156.4, 138.5, 138.23, 138.17, 137.7, 136.6, 135.7, 128.53, 128.50, 128.46, 128.45 (2C), 128.3, 128.10, 128.07, 127.96, 127.85, 127.81, 127.78, 127.76, 127.70, 127.6, 119.4, 104.0, 84.2, 81.7, 79.5, 75.3, 74.8, 74.0, 73.7, 71.9, 70.6, 70.2, 68.4, 66.6, 52.0, 40.9, 36.2, 29.6, 26.2, 25.0; ESI HRMS: $m/z$: calcd for $C_{53}H_{62}N_2O_{10}Na$: 909.4297; found: 909.4298 $[M+Na^+]$; elemental analysis calcd (%) for $C_{53}H_{62}N_2O_{10}$: C 71.77, H 7.04, N 3.16; found: C 71.73, H 6.71, N 2.96.

***S*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-D-galactopyranose (25)**: Disaccharide **19** (0.950 g, 1.05 mmol), palladium chloride (0.265 g, 1.49 mmol, 1.4 equiv), and sodium acetate (0.360 g, 4.39 mmol, 4.2 equiv) were combined and added to acetic acid/water 19:1 (30 mL). The reaction was immersed in an ultrasonic cleaning bath (Branson model B-32) and water temperature was maintained below 30 °C by portionwise addition of ice. After 5 h, the mixture was filtered through Celite, diluted with ethyl acetate (80 mL), washed with an equal volume of water, dried over sodium sulfate, filtered and concentrated. The residue was chromatographed by using hexanes/ethyl acetate/acetone 3:3:2 as eluent to yield **25** (0.735 g, 0.85 mmol, 81 %) as an inseparable mixture of α and β isomers. ESI HRMS: $m/z$: calcd for $C_{40}H_{47}NO_{18}SNa$: 884.2406; found: 884.2405 $[M+Na^+]$.

***S*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-D-galactopyranosyl trichloroacetimidate (4)**: Hemiacetal **25** (0.560 g, 0.65 mmol) was concentrated from toluene (2x) and dried in vacuo. The residue was then taken up in dichloromethane (10.0 mL) and then trichloroacetonitrile (1.0 mL, 10 mmol, 15.4 equiv) and 1,8-diazabicyclo-[5.4.0]-undec-7-ene (0.050 mL, 0.33 mmol, 0.5 equiv) were added. After 2 h, the reaction mixture was concentrated and the residue chromatographed on silica gel with ethyl acetate/hexanes 7:1 containing triethylamine (2 %) as eluent to afford **4** (0.458 g, 0.46 mmol, ~70 %) and some minor contaminants (<10 %). ESI HRMS: $m/z$: calcd for $C_{42}H_{47}N_2O_{18}SCl_3Na$: 1027.1505; found: 1027.1502 $[M+Na^+]$.

***S*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-3-*O*-benzyl-pent-4-ene-1,3-diol (3a)**: Acceptor **5** (0.110 g, 0.17 mmol) and imidate **4** (0.250 g, 0.25 mmol, 1.5 equiv) were combined and concentrated from toluene at room temperature. The residue was dissolved in freshly distilled dichloromethane (12 mL) containing powdered 4 Å molecular sieves, and the mixture was stirred under argon for 45 min at room temperature before being cooled to 0 °C. $BF_3{\cdot}Et_2O$ (0.030 mL, 0.24 mmol, 1.5 equiv) was added and the reaction mixture was allowed to warm to room temperature. After 1.5 h, an additional 0.5 equivalents of $BF_3{\cdot}Et_2O$ was added and stirring was continued for 2 h before the mixture was made basic with triethylamine, filtered, and concentrated. Chromatography of the residue on silica gel with a gradient of hexanes/ethyl acetate/acetone (8:6:1→8:6:2) afforded **3a** (0.158 g, 0.10 mmol, 64 %). $[\alpha]_D = 57$ ($c = 0.40$, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$): $\delta = 8.22$–8.19 (m, 2H, Ar), 7.57–7.53 (m, 1H, Ar), 7.51–7.45 (m, 4H, Ar), 7.35–7.30 (m, 5H, Ar), 7.28–7.15 (m, 18H, Ar), 5.77 (ddd, 1H, $J=8.0$, 10.4, 17.4 Hz, H-$4_{sph}$), 5.69 (ddd, 1H, $J_{8'',9a''}=2.5$, $J_{8'',9b''}=6.1$, $J_{8'',7''}=10.0$ Hz, H-8''), 5.39 (s, 1H, CHPh), 5.35–5.32 (m, 1H, H-$5a_{sph}$), 5.29–5.23 (m, 3H, H-1', H-2', H-$5b_{sph}$), 5.22 (dd, 1H, $J_{7'',6''}=2.3$ Hz, H-7''), 5.12 (d, 1H, $J=11.7$ Hz, $CH_2Ph$), 5.00 (d, 1H, $J_{NH,5''}=10.1$ Hz, NH), 4.89 (d, 1H, $J=11.7$ Hz, $CH_2Ph$), 4.79 (ddd, 1H, $J_{4'',5''}=11.4$, $J_{4'',3''ax}=12.8$, $J_{4'',3''eq}=4.6$ Hz, H-4''), 4.76 (d, 1H, $J=11.3$ Hz, $CH_2Ph$), 4.63 (d, 1H, $J=11.3$ Hz, $CH_2Ph$), 4.55 (d, 1H, $J=11.9$ Hz, $CH_2Ph$), 4.35 (dd, 1H, $J_{9a'',9b''}=12.5$ Hz, H-9a''), 4.31 (d, 1H, $J=11.9$ Hz, $CH_2Ph$), 4.28 (d, 1H, $J_{1,2}=7.8$ Hz, H-1), 4.18 (d, 1H, $CH_2Ph$), 4.15 (d, 1H, $CH_2Ph$), 4.05 (dd, 1H, H-9b''), 4.01 (dd, 1H, $J_{6a',6b'}=12.5$, $J_{6a',5'}$ ~0.5 Hz, H-6a'), 3.94–3.87 (m, 4H, H-4, H-$1a_{sph}$, H-5'', H-$3_{sph}$), 3.84 (dd, 1H, $J_{3',2'}=10.7$, $J_{3',4'}=3.2$ Hz, H-3'), 3.78–3.69 (m, 3H, H-6b', H-$2_{sph}$, H-6''), 3.74 (s, 3H, $CO_2CH_3$), 3.66 (dd, 1H, $J_{6a,6b}=10.7$ Hz, H-6a), 3.64 (dd, 1H, $J_{3,2}=J_{3,4}=8.8$ Hz, H-3), 3.61 (d, 1H, H-4'), 3.57–3.53 (m, 2H, H-5', H-$1b_{sph}$), 3.43 (dd, 1H, $J_{6b,5}=6.8$ Hz, H-6b), 3.40–3.34 (m, 2H, H-2, H-5), 2.56 (dd, 1H, $J_{3eq,3ax}=12.8$ Hz, H-3''$_{eq}$), 2.18 (s, 3H, $C(O)CH_3$), 2.04 (s, 3H, $C(O)CH_3$), 1.96 (s, 3H, $C(O)CH_3$), 1.87 (dd, 1H, H-3''$_{ax}$), 1.84 (s, 3H, $C(O)CH_3$), 1.73 (s, 3H, $C(O)CH_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta = 170.8$, 170.7, 170.3, 170.1, 170.0, 169.7, 165.6, 139.3, 138.7, 138.4, 137.9, 137.8, 134.1, 133.1, 130.27, 130.25, 128.7, 128.5, 128.3, 128.2, 128.1, 128.00, 127.94, 127.87, 127.6 (2C), 127.4, 127.18, 127.17, 127.16, 126.7, 126.2, 120.7, 103.1, 101.9, 101.2, 83.2, 82.0, 81.2, 79.9, 77.7, 75.7, 75.0, 74.68, 74.66, 73.6, 72.8, 70.4, 69.8, 69.6, 69.4, 68.7, 68.3, 68.1, 67.1, 67.0, 64.2, 62.6, 53.0, 49.2, 46.0, 37.5, 23.2, 21.5, 20.84, 20.77, 20.68; ESI HRMS: $m/z$: calcd for $C_{79}H_{88}N_4O_{24}S$ Na: 1531.5406; found: 1531.5407 $[M+Na^+]$; elemental analysis calcd (%) for $C_{79}H_{88}N_4O_{24}S$: C 62.85, H 5.88, N 3.71; found: C 62.61, H 6.02, N 3.55.

***S*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(*N*-carboxybenzyl-6-aminohexanamido)-3-*O*-benzyl-pent-4-ene-1,3-diol (26)**: Azide **3a** (0.150 g, 0.10 mmol) was dissolved in pyridine and water 9:1 (10 mL) then triphenylphosphine (0.052 g, 0.20 mmol, 2 equiv) was added and the mixture was heated at 50 °C for 4 h before being allowed to cool to room temperature. 6-(Benzyloxycarbonylamino)hexanoic acid succinimidyl ester (0.180 g, 0.50 mmol, 5 equiv) was added and the solution was heated at 50 °C overnight and then concentrated. Chromatography of the residue on silica gel using hexanes-ethyl acetate-acetone (4:3:2→3:3:2) as eluent afforded **26** (0.155 g, 0.09 mmol, 90 %). $[\alpha]_D = 53$ ($c = 0.41$, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$): $\delta = 8.21$–8.18 (m, 2H, Ar), 7.58–7.54 (m, 1H, Ar), 7.51–7.45 (m, 4H, Ar), 7.36–7.12 (m, 28H, Ar), 5.80 (d, 1H, $J_{NH,H2sph}=8.9$ Hz, $NH_{sph}$), 5.70–5.62 (m, 2H, H-8'', H-$4_{sph}$), 5.41 (s, 1H, CHPh),


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5.29–5.24 (m, 2H, H-1′, H-2′), 5.23 (dd, 1H, $J_{7'',8''}=2.3$, $J_{7'',6''}=10.1$ Hz, H-7″), 5.19 (dd, 1H, $J=10.4$, 1.5 Hz, H-5a$_{sph}$), 5.16 (d, 1H, $J=11.4$ Hz, C$H_2$Ph), 5.12–5.06 (m, 2H, C$H_2$Ph, H-5b$_{sph}$), 5.01 (d, 1H, $J_{NH,H5''}=10.0$ Hz, N*H*Ac), 4.85 (d, 1H, $J=11.4$ Hz, C$H_2$Ph), 4.82–4.75 (m, 2H, H-4″, N*H*Z), 4.68 (d, 1H, C$H_2$Ph), 4.63 (d, 1H, C$H_2$Ph), 4.54 (d, 1H, $J=11.9$ Hz, C$H_2$Ph), 4.34 (dd, 1H, $J_{9a'',8''}=2.6$, $J_{9a'',9b''}=12.4$ Hz, H-9a″), 4.30 (d, 1H, $J=11.9$ Hz, C$H_2$Ph), 4.24 (brs, 2H, C$H_2$Ph), 4.21 (d, 1H, $J_{1,2}=7.9$ Hz, H-1), 4.17 (dd, 1H, $J_{1a,2}=4.6$, $J_{1a,1b}=10.9$ Hz, H-1a$_{sph}$), 4.11–4.06 (m, 2H, H-2$_{sph}$, H-6a′), 4.05 (dd, 1H, $J_{9''b,8''}=6.6$ Hz, H-9b″), 4.00 (dd, 1H, $J_{4,3}=8.8$, $J_{4,5}=9.6$ Hz, H-4), 3.92–3.84 (m, 2H, H-5″, H-3′), 3.83–3.78 (m, 2H, H-6b′, H-3$_{sph}$), 3.75–3.72 (m, 1H, H-6″), 3.74 (s, 3H, $CO_2CH_3$), 3.67 (dd, 1H, $J_{6a,5}=1.6$, $J_{6a,6b}=10.9$ Hz, H-6a), 3.64 (brd, 1H, H-4′), 3.63 (dd, 1H, $J_{3,2}=9.0$ Hz, H-3), 3.59 (brs, 1H, H-5′), 3.56 (dd, 1H, $J_{1b,2}=6.3$ Hz, H-1b$_{sph}$), 3.52 (dd, 1H, $J_{6b,5}=6.3$ Hz, H-6b), 3.34 (dd, 1H, H-2), 3.30 (m, 1H, H-5), 3.10 (m, 2H, C$H_2$NHZ), 2.57 (dd, 1H, $J_{3''eq,4''}=4.6$, $J_{3''eq,3''ax}=12.8$ Hz, H-3″$_{eq}$), 2.16, 2.05, 1.97, (3×s, 3×3H, C(O)C$H_3$), 1.88 (dd, 1H, $J_{''ax,4''}=12.0$ Hz, H-3″$_{ax}$), 1.85–1.68 (m, 2H, C$H_2$), 1.84, 1.73, (2×s, 3×3H, C(O)C$H_3$), 1.42–1.34 (m, 4H, 2×C$H_2$), 1.16–1.08 (m, 2H, C$H_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 172.3, 170.8, 170.7, 170.2, 170.1, 170.0, 169.7, 165.6, 156.3, 139.1, 138.5, 138.3, 138.2, 137.7, 136.7, 135.7, 133.1, 130.23, 130.21, 128.7, 128.489, 128.486, 128.478, 128.31, 128.29, 128.14, 128.13, 128.02, 128.00, 127.8, 127.7, 127.57, 127.53, 127.50, 127.3, 127.1, 126.9, 126.2, 119.3, 103.8, 101.5, 101.2, 83.4, 81.9, 81.2, 79.4, 75.6, 75.2, 74.8, 74.5, 73.6, 72.7, 70.5, 69.8, 69.5, 69.4, 68.8, 68.5, 68.1, 67.1, 66.9, 66.5, 62.5, 53.0, 52.2, 49.2, 45.9, 40.8, 37.5, 36.0, 29.7, 29.6, 26.2, 24.9, 23.2, 21.5, 20.9, 20.8, 20.7; ESI HRMS: *m/z*: calcd for $C_{93}H_{107}N_3O_{29}SNa$: *m/z*: 1752.6714; found: 1752.6710 [$M$+Na$^+$]; elemental analysis calcd (%) for $C_{93}H_{107}N_3O_{29}S$: C 64.53, H 6.23, N 2.43; found: C 64.54, H 6.39, N 2.25.

***S*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-(4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(*N*-carboxybenzyl-6-aminohexanamido)-3-*O*-benzylpent-4-ene-1,3-diol (29)**: Trisaccharide **26** (0.140 g, 0.08 mmol) was combined with methanol (7.0 mL) and a small piece of sodium was added. After evolution of gas had ceased, the mixture was heated at 55 °C for 7 h and then cooled to room temperature. Water (0.350 mL) was added and the solution was heated at 40 °C overnight. After acidification with acetic acid, the reaction mixture was concentrated and the residue chromatographed on silica gel using a gradient of methanol and dichloromethane (0:100→25:75). The residue was dissolved in 50 % methanol and loaded on a SepPak cartridge (5 g), then eluted with 80–90 % methanol to afford **29** (0.108 g, 0.08 mmol, 94 %). $[\alpha]_D$ = 10 ($c$ = 0.34, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ = 7.43–7.38 (m, 4H, Ar), 7.36–7.34 (m, 2H, Ar), 7.33–7.28 (m, 10H, Ar), 7.28–7.20 (m, 11H, Ar), 7.10–7.04 (m, 3H, Ar), 5.74 (ddd, 1H, H-4$_{sph}$), 5.50 (s, 1H, C*H*Ph), 5.30–5.24 (m, 2H, H-5a$_{sph}$, H-5b$_{sph}$), 5.16 (d, 1H, $J=10.7$ Hz, C$H_2$Ph), 5.03 (brs, 2H, $CH_2$Ph), 4.78 (d, 1H, $J=11.4$ Hz, C$H_2$Ph), 4.63 (brd, 3H, 3×C$H_2$Ph), 4.58–4.54 (m, 3H, C$H_2$Ph, C$H_2$Ph, H-1′), 4.43 (d, 1H, $J_{1,2}=7.8$ Hz, H-1), 4.38 (d, 1H, $J_{1,2}=11.8$ Hz, C$H_2$Ph), 4.17 (ddd, 1H, $J_{1a,2}=6.4$, $J=6.6$, 3.8 Hz, H-2$_{sph}$), 4.12 (dd, 1H, $J_{1a,1b}=10.1$ Hz, H-1a$_{sph}$), 4.09 (dd, 1H, $J_{4,3}=3.5$, $J_{4,5}=0.6$ Hz, H-4′), 4.06 (dd, 1H, $J_{6a',5'}=1.4$, $J_{6a',6b'}=12.4$ Hz, H-6a′), 4.04–3.99 (m, 3H, H-8″, H-4, H-6a), 3.95 (dd, 1H, $J_{6a,5}=1.6$, $J_{6a,6b}=11.0$ Hz, H-6b), 3.93–3.87 (m, 2H, H-3$_{sph}$, H-6b′), 3.82 (dd, 1H, $J_{9a'',8''}=2.8$, $J_{9a'',9b''}=11.4$ Hz, H-9a″), 3.77–3.73 (m, 3H, H-1b$_{sph}$, H-5″, H-4″), 3.69 (dd, 1H, $J_{9b'',8''}=4.4$ Hz, H-9b″), 3.65 (dd, 1H, $J_{3',4'}=3.4$, $J_{3',2'}=11.0$ Hz, H-3′), 3.62 (dd, 1H, $J_{3,2}=J_{3,4}=9.1$ Hz, H-3), 3.59 (dd, 1H, $J=1.8$, 9.2 Hz, H-7″), 3.56–3.52 (m, 2H, H-6″, H-5), 3.47 (dd, 1H, $J_{2',1'}=7.6$, $J_{2',3'}=11.0$ Hz, H-2′), 3.29 (m, 1H, H-2), 3.17 (brs, 1H, H-5′), 3.01 (m, 2H, C$H_2$NHZ), 2.94 (m, 1H, H-3″$_{eq}$), 2.00 (s, 3H, NC(O)C$H_3$), 2.00 (m, 2H, C$H_2$), 1.70 (m, 1H, H-3″$_{ax}$), 1.47 (m, 2H, C$H_2$), 1.39 (m, 2H, C$H_2$), 1.21 (m, 2H, C$H_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 178.6, 175.7, 175.4, 175.0, 140.2, 140.1, 140.0, 139.9, 139.8, 139.6, 136.9, 129.8, 129.6, 129.45, 129.39, 129.34, 129.28, 129.09, 129.03, 128.99, 128.98, 128.93, 128.85, 128.81, 128.7, 128.6, 128.5, 128.3, 127.4, 120.1, 105.3, 104.7, 102.2, 85.8, 84.6, 83.0, 80.8, 78.1, 77.6, 76.9, 76.0, 75.9, 74.2, 72.5, 71.8, 70.8, 70.4, 70.0, 69.81, 69.78, 69.6, 69.1, 67.3, 64.4, 54.0, 53.7, 50.9, 49.3, 42.9, 41.7, 37.1, 30.6, 27.3, 26.6, 22.6; ESI HRMS: *m/z*: calcd for $C_{77}H_{93}N_3O_{22}SNa$: 1466.5868; found: 1466.5869 [$M$+Na$^+$].

***S*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexanamido)-pent-4-ene-1,3-diol (1)**: Trisaccharide **29** (0.115 g, 0.08 mmol) was dissolved in anhydrous tetrahydrofuran (8.0 mL) and added via cannula to a solution of sodium (0.090 g) in liquid ammonia at −78 °C. Sodium metal (0.060 g) was added, and the dark blue solution was stirred for 1 h, then quenched with methanol (15 mL) and acetic acid (0.50 mL). The mixture was allowed to warm to room temperature and was stirred overnight to allow ammonia to evaporate. The methanolic solution was combined with Iatrobeads (~1 g) and concentrated. The solid material was applied to the top of a column of Iatrobeads, and eluted by application of a linear gradient of methanol/isopropanol/water 1:1:0→41:41:18. HPLC purification (Hamilton PRP column; water/methanol gradient) of the material obtained yielded **1** (0.0571 g, 0.07 mmol, 83 %). $[\alpha]_D$ = 33 ($c$ = 1.0, $H_2O$); $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.85 (ddd, 1H, $J_{4,3}=7.0$, $J_{4,5a}=17.1$, $J_{4,5b}=10.4$ Hz, H-4$_{sph}$), 5.32 (brd, 1H, H-5a$_{sph}$), 5.26 (brd, 1H, H-5b$_{sph}$), 4.53 (d, 1H, $J_{1',2'}=7.2$ Hz, H-1′), 4.47 (d, 1H, $J_{1,2}=8.0$ Hz, H-1), 4.24–4.20 (m, 1H, H-3$_{sph}$), 4.11 (ddd, 1H, $J_{2,1a}=6.7$, $J_{2,1b}=3.6$, $J_{2,3}=3.6$ Hz, H-2$_{sph}$), 4.04 (dd, 1H, $J_{1a,1b}=10.5$ Hz, H-1a$_{sph}$), 4.00 (dd, 1H, $J_{6a,6b}=12.3$, $J_{6a,5}=2.2$ Hz, H-6a), 3.92 (dd, 1H, $J=2.5$, 6.2, 9.2 Hz, H-8″), 3.88–3.82 (m, 4H, H-4′, H-9a″, H-5″, H-6b), 3.79 (dd, 1H, H-1b), 3.78–3.75 (m, 1H, H-5′), 3.74–3.70 (m, 2H, H-6a′, H-6b′), 3.70–3.63 (m, 3H, H-4, H-4″, H-7″), 3.64 (dd, 1H, $J\sim J\sim 8.7$ Hz, H-3), 3.60–3.56 (m, 3H, H-6″, H-5, H-9b″), 3.39 (dd, 1H, $J_{2',3'}=11.4$ Hz, H-2′), 3.37 (dd, 1H, $J_{3',4'}=2.5$ Hz, H-3′), 3.32 (dd, 1H, H-2), 3.00–2.96 (m, 2H, C$H_2$NH$_2$), 2.80 (dd, 1H, $J_{3eq,3ax}=12.7$, $J_{3''eq,4''}=4.8$ Hz, H-3eq″), 2.28 (dd, 2H, NC(O)C$H_2$), 2.02 (s, 3H, NHC(O)C$H_3$), 1.81 (dd, 1H, $J_{3ax,4''}=11.3$ Hz, H-3″$_{ax}$), 1.70–1.58 (m, 4H, 2×C$H_2$), 1.41–1.34 (m, 2H, C$H_2$); $^{13}$C NMR (125 MHz, $D_2O$): $\delta$ = 177.6, 175.9, 175.4, 137.2, 118.9, 105.0, 103.2, 85.0, 79.1, 78.7, 75.9, 75.7, 75.1, 73.8, 72.82, 72.77, 69.7, 69.5, 69.35 (2C), 69.0, 63.5, 62.1, 60.9, 53.9, 52.5, 51.5, 41.5, 40.2, 36.4, 27.4, 26.0, 25.6, 22.9; ESI HRMS: *m/z*: calcd for $C_{34}H_{59}N_3O_{20}SNa$: 884.3316; found: 884.3310 [$M$+Na$^+$].

***S*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-[2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)]-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexanamido)-pent-4-ene-1,3-diol (2)**: UDP-*N*-acetylglucosamine disodium salt (0.022 g, 0.02 mmol, 1.5 equiv) was added to a falcon tube containing **1** (15.2 mg, 18 μmol, 1.0 equiv). Freshly prepared β(1,4) *N*-acetylgalactosaminyl transferase (2.0 mL) in 50 mM HEPES, pH 7.5 containing 10 % glycerol (v/v) and UDP *N*-acetylglucosamine 4-epimerase (2.0 mL) in 0.2 M NaCl, 1.0 mM EDTA, 20 mM HEPES, 5 mM β-mercaptoethanol, pH 7.0 were added to the mixture, followed by 4.0 μL of 0.5 M manganese(II) chloride and 12 μL (12 units) of alkaline phosphatase. The mixture was tumbled a total of 24 h and then centrifuged for 30 min at 14000 rpm. The supernatant was applied to a SepPak cartridge (5 g) which had been conditioned with methanol (10 mL) followed by 1 % ammonia solution (20 mL). After application of the sample, the cartridge was washed with 1 % ammonia (25 mL) and eluted with 10 % methanol containing 1 % ammonia. HPLC of the residue thus obtained (Hamilton PRP-1 semi-preparative column; water/methanol gradient elution) afforded the desired $GM_2$ analogue **2** (18.3 mg, 0.017 mmol, 94 %). $[\alpha]_D$ = 22 ($c$ = 0.20, $H_2O$); $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.85 (ddd, 1H, $J_{4,3}=7.0$, $J_{4,5a}=17.3$, $J_{4,5b}=10.5$ Hz, H-4$_{sph}$), 5.35–5.30 (m, 1H, H-5a$_{sph}$), 5.28–5.25 (m, 1H, H-5b$_{sph}$), 4.75 (obscured, 1H, H-1‴), 4.53 (d, 1H, $J_{1',2'}=7.6$ Hz, H-1′), 4.47 (d, 1H, $J_{1,2}=7.9$ Hz, H-1), 4.24–4.20 (m, 1H, H-3$_{sph}$), 4.11 (ddd, 1H, $J_{2,1a}=6.6$, $J_{2,1b}=3.6$, $J_{2,3}=3.6$ Hz, H-2$_{sph}$), 4.06 (dd, 1H, $J_{1a,1b}=10.6$ Hz, H-1a$_{sph}$), 4.01–3.98 (m, 2H, H-4′, H-6a), 3.92–3.55 (m, 21H, H-3, H-4, H-5, H-6b, H-5′, H-6a′, H-6b′, H-4″, H-5″, H-6″, H-7″, H-8″, H-9a″, H-9b″, H-2‴, H-3‴, H-4‴, H-5‴, H-6a‴, H-6b‴, H-1b$_{sph}$), 3.42 (dd, 1H, $J_{3',2'}=11.5$, $J_{3',4'}=2.4$ Hz, H-3′), 3.32 (dd, 1H, $J_{2,3}=9.1$ Hz, H-2), 3.21 (dd, 1H, H-2′), 3.00–2.97 (m, 2H, C$H_2$NH$_2$), 2.80 (dd, 1H, $J_{3eq,3ax}=12.6$, $J_{3''eq,4''}=4.8$ Hz, H-3″$_{eq}$), 2.28 (dd, 2H, NC(O)C$H_2$), 2.09 (s, 3H, NHC(O)C$H_3$), 2.03 (s, 3H, NHC(O)C$H_3$), 1.84 (dd, 1H, $J_{3ax,4''}=11.6$ Hz, H-3″$_{ax}$), 1.70–1.59 (m, 4H, 2×C$H_2$), 1.40–1.35 (m, 2H, C$H_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 177.6, 175.8, 175.5, 175.3, 137.2, 118.9, 105.0, 104.1, 103.2, 84.6, 79.2, 77.6, 77.0, 75.72, 75.69, 75.5, 75.0, 73.7, 73.0, 72.7, 71.5, 69.44, 69.37, 69.2, 69.1, 68.6, 63.5, 61.9, 61.6, 60.9, 53.8, 53.4, 52.5, 50.5, 41.5, 40.2, 36.4, 27.4, 25.9, 25.6, 23.5, 22.9; ESI HRMS: *m/z*: calcd for $C_{42}H_{72}N_4O_{25}SNa$: 1065.428417; found: 1065.428462 [$M$+Na$^+$].

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**5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid-(2→8)-[*S*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)]-(2→3)-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexanamido) -pent-4-ene-1,3-diol (30)**: $GM_3$ analogue **1** (2.09 mg, 2.4 μmol) and crude CMP-Neu5Ac (21 mg, 7.3 μmol, 3.0 equiv) were combined as solids. To this mixture was added successively freshly prepared α(2–3/8) sialyltransferase in 20 mM Tris buffer, pH 8.3 (2.0 mL, 1.33 U mL$^{-1}$), $MnCl_2$ (20 μL of a 0.5 M solution), and alkaline phosphatase (2 μL; 2U). The mixture was tumbled overnight at room temperature before centrifugation (30 min at 14000 rpm). The supernatant was loaded directly onto a Biogel P4 column (2×50 cm) and eluted with 5% methanol at a flow rate of 0.4 mLmin$^{-1}$. Fractions containing the tetrasaccharide were purified further by HPLC (Hamilton PRP-1 semipreparative column, water-methanol gradient) to afford **30** (1.1 mg, 1.0 μmol, 39%): $^1$H NMR (500 MHz, $D_2O$): $\delta$ = [note: α(2→3) and α(2→8) linked Neu5Ac residues could not be unambiguously identified, but individual spin systems are denoted by $H_{\#}$ and H*) 5.86 (ddd, 1H, $J$=6.9, 10.5, 17.2 Hz, H-4$_{sph}$), 5.33 (ddd, 1H, $J$=1.2, 1.2, 17.2 Hz, H-5a$_{sph}$), 5.28 (ddd, 1H, H-5b$_{sph}$), 4.54 (d, 1H, $J_{1',2'}$=7.0 Hz, H-1′), 4.48 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.25 (dd, 1H, $J_{9a*,8*}$= 4.1, $J_{9a*,9b*}$=12.4 Hz, H-9a*), 4.24–4.21 (m, 1H, H-3$_{sph}$), 4.17–4.10 (m, 2H, H-8*, H-2$_{sph}$), 4.07–4.01 (m, 2H, H-1a$_{sph}$, H-6a), 3.95–3.89 (m, 1H, H-8$_{\#}$), 3.89–3.78 (m, 8H, H-9a$_{\#}$, H-4′, H-6b, H-5*, H-7*, H-1b$_{sph}$, H-5$_{\#}$, H-5′), 3.74–3.53 (m, 12H, H-6a′, H-6b′, H-4, H-4*, H-9b*, H-3, H-9b$_{\#}$, H-5, H-6$_{\#}$, H-6*, H-7$_{\#}$, H-4$_{\#}$), 3.42 (dd, 1H, $J_{3',4'}$=2.3, $J_{3',2'}$=11.5 Hz, H-3′), 3.38 (dd, 1H, H-2′), 3.33 (dd, 1H, $J_{2,3}$=8.9 Hz, H-2), 3.02–2.97 (m, 2H, C$H_2$NH$_2$), 2.78 (dd, 1H, $J_{3eq*,4*}$=4.7, $J_{3eq*,3ax*}$=12.3 Hz, H-3eq*), 2.75 (dd, 1H, $J_{3eq\#,4\#}$=4.3, $J_{3eq\#,3ax\#}$=12.3 Hz, H-3eq$_{\#}$), 2.32–2.27 (m, 2H, C(O)C$H_2$), 2.07 (s, 3H, NHC(O)CH$_3$), 2.04 (s, 3H, NHC(O)CH$_3$), 1.77 (dd, 1H, $J_{3ax\#,4\#}$=12.0 Hz, H-3ax$_{\#}$), 1.73 77 (dd, 1H, $J_{3ax*,4*}$=12.1 Hz, H-3ax*), 1.70–1.59 (m, 4H), 1.43–1.36 (m, 2H); ESI HRMS: $m/z$: calcd for $C_{45}H_{75}N_4O_{28}SNa_3$: 1220.3971; found: 610.1989 $[M+2Na]^{2+}$.

***p*-Nitrophenyl ester of 1 (34)**: Amine **1** (10.5 mg, 0.01 mmol) was dissolved in *N*,*N*-dimethylformamide (1.5 mL) and triethylamine (3.7 μL; 0.02 mmol, 2.2 equiv). The *p*-nitrophenyl diester **33** (0.047 g, 0.12 mmol, 10 equiv) was added, and reaction progress was monitored by TLC (dichloromethane/methanol/water/acetic acid 4:5:1:0.5). After 90 min, several drops of acetic acid were added, and the mixture was concentrated at room temperature from toluene (3×) on a rotary evaporator. The residue was suspended in 2% acetic acid solution (3 mL) and filtered through cotton. The filtrate was applied to a SepPak cartridge (0.4 g), washed with 2% acetic acid (10.0 mL), and eluted with 2% acetic acid in methanol. Concentration of the eluent yielded a clear solid which was purified by HPLC (Hamilton PRP-1 semi-preparative column; water-methanol gradient elution) to yield the activated oligosaccharide **34** (0.011 g, 10 μmol, 81%). $^1$H NMR spectra were acquired in $CD_3OD$. Although visible, resonances corresponding to the Neu5Ac residue were broadened significantly. Selected $^1$H NMR resonances are reported here (600 MHz, $CD_3OD$): $\delta$ = 8.29 (m, 2H, Ar), 8.14 (brs, 1H, N$H$), 7.94 (brs, 1H, N$H$), 7.89 (brs, 1H, N$H$), 7.37 (m, 2H, Ar), 5.86 (ddd, 1H, $J$=6.7, 10.5, 17.1 Hz, H-4$_{sph}$), 4.45 (d, 1H, $J_{1',2'}$=7.1 Hz, H-1′), 4.29 (dd, 1H, $J_{1,2}$= 7.8 Hz, H-1), 1.99 (brs, 3H, NHC(O)C$H_3$); ESI HRMS: $m/z$: calcd for $C_{46}H_{70}N_4O_{25}SNa$: 1133.3948; found: 1133.3948 $[M+Na^+]$. [Note: addition of an amine (for example diallylamine) to the NMR tube resulted in resolution of the $^1$H NMR signals corresponding to the Neu5Ac residue].

***p*-Nitrophenyl ester of 2 (35)**: Amine **2** (4.5 mg, 4.2 μmol) was dissolved in *N*,*N*-dimethylformamide (1.5 mL) and triethylamine (2.0 μL, 9.2 μmol, 2.2 equiv). The *p*-nitrophenyl diester **33** (0.017 g, 42 μmol, 10 equiv) was added, and reaction progress was monitored by TLC (dichloromethane/methanol/water/acetic acid 4:5:1:0.5). After 2 h, several drops of acetic acid were added, and the mixture was concentrated at room temperature from toluene (3×) on a rotary evaporator. The residue was suspended in 2% acetic acid solution (4 mL) and filtered through cotton. The filtrate was applied to a SepPak cartridge (0.4 g), washed with 2% acetic acid (5 mL), and eluted with 2% acetic acid in methanol. Concentration of the eluent yielded a clear solid that was purified by HPLC (Hamilton PRP-1 semi-preparative column; water-methanol gradient elution) to yield the activated thiotetrasaccharide **35** (0.0031 g, 2.3 μmol, 56%): $^1$H NMR spectra were acquired in $CD_3OD$. Although visible, resonances corresponding to the Neu5Ac residue were broadened significantly. Selected $^1$H NMR resonances are reported here (600 MHz, $CD_3OD$): $\delta$ = 8.29 (m, 2H, Ar), 8.06 (brd, 1H, N$H$), 7.94 (brt, 1H, N$H$), 7.89 (d, 1H, N$H$), 7.37 (m, 2H, Ar), 5.86 (ddd, 1H, $J$=6.6, 10.6, 17.3 Hz, H-4$_{sph}$), 4.63 (brs, 1H, H-1″), 4.43 (d, 1H, $J_{1',2'}$=7.2 Hz, H-1′), 4.29 (d, 1H, $J_{1,2}$= 7.7 Hz, H-1), 1.98 (brs, 3H, NHC(O)C$H_3$); ESI HRMS: $m/z$: calcd for $C_{46}H_{70}N_4O_{25}SNa$: 1336.4741; found: 1336.4742 $[M+Na^+]$.

**β-D-Glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexanamido)-pent-4-ene-1,3-diol (43)**: Glucoside **24** (0.068 g, 0.08 mmol) in tetrahydrofuran (6 mL) was added to a solution of sodium (0.090 g) in ammonia (50 mL) at −78 °C. After 1 h, methanol (5 mL) and acetic acid (0.5 mL) were added, and ammonia was removed by evaporation with a stream of argon. The residue was concentrated and dissolved in water, then applied to a SepPak cartridge (5 g) that had been preconditioned with methanol followed by 1% ammonia. After washing with 1% ammonia, the product was eluted with 20% methanol containing the same and concentrated. The residue was purified by HPLC (Hamilton PRP-1 column, water-methanol gradient) to afford **43** (0.026 g, 0.07 mmol, 85%). $[\alpha]_D$ = −1 ($c$ = 0.44, $CHCl_3$); $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ = 5.87 (ddd, 1H, $J$= 6.6, 10.4, 17.1 Hz, H-4$_{sph}$), 5.27 (ddd, 1H, $J$=1.5, 1.6, 17.1 Hz, H-5a$_{sph}$), 5.14 (ddd, 1H, $J$=1.2, 1.6, 10.4 Hz, H-5b$_{sph}$), 4.26 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 4.16–4.13 (m, 1H, H-3$_{sph}$), 4.10 (dd, 1H, $J_{1a,1b}$=10.2, $J_{1a,2}$=5.7 Hz, H-1a$_{sph}$), 4.04 (ddd, 1H, $J_{2,1b}$=3.4, $J_{2,3}$=7.4 Hz, H-2$_{sph}$), 3.87 (dd, 1H, $J_{6a,5}$= 1.7, $J_{6a,6b}$=11.8 Hz, H-6a), 3.68–3.64 (m, 1H, H-6b), 3.65 (dd, 1H, H-1b$_{sph}$), 3.37–3.30 (m, 1H, H-3), 3.28–3.25 (m, 2H, H-4, H-5), 3.18 (dd, 1H, H-2), 2.89 (m, 2H, CH$_2$), 2.23 (m, 2H, CH$_2$), 1.70–1.60 (m, 4H, CH$_2$, CH$_2$), 1.40 (m, 2H, CH$_2$); $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 175.8, 139.5, 117.0, 104.6, 78.01, 77.95, 75.2, 73.3, 71.6, 69.6, 62.6, 54.7, 40.5, 36.7, 28.3, 26.9, 26.2; ESI HRMS: $m/z$: calcd for $C_{17}H_{33}N_2O_8$: 393.22314; found: 393.22307 $[M+Na^+]$.

**6-Azidohexyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (44)**: 6-Azidohexyl β-D-galactopyranoside[79] (0.017 g, 0.06 mmol) was combined with crude CMP-Neu5Ac (crude mass 180 mg, estimate 0.09 mmol, 1.5 equiv). α(2,3) Sialyl transferase (CST-04 in 50 mM HEPES, pH 7.5, 10% glycerol (v/v); 0.14 U mL$^{-1}$; 4.75 mL), $MnCl_2$ and $MgCl_2$ (100 μL of a 0.5 M solution; final conc. 10 mM), dithiothreitol (38 μL, 0.4 M; final conc. 3 mM) and alkaline phosphatase (12 μL) were added and the mixture was tumbled overnight. After centrifugation (30 min at 14000 rpm) the supernatant was loaded onto a SepPak cartridge (5 g) and eluted with a gradient of water and methanol to afford **44** (0.0311 g, 0.05 mmol, 94%). $[\alpha]_D$ = −3 ($c$ = 0.52, $H_2O$); $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.46 (d, 1H, $J_{1,2}$= 8.0 Hz, H-1), 4.08 (dd, 1H, $J_{3,2}$=9.8, $J_{3,4}$=3.3 Hz, H-3), 3.95 (d, 1H, $J_{3,4}$= 3.3 Hz, H-4), 3.94–3.85 (m, 3H, OCH$_2$, H-8′, H-9a′), 3.84 (dd, 1H, $J_{5',6'}$= $J_{5',6'}$=10.1 Hz, H-5′), 3.77–3.72 (m, 2H, H-6a, H-6b), 3.72–3.61 (m, 5H, H-4′, H-5, OC$H_2$, H-6′, H-9b′), 3.59 (dd, 1H, $J$=1.8, 9.1 Hz, H-7′), 3.53 (dd, 1H, H-2), 3.32 (dd, 2H, C$H_2$N$_3$), 2.76 (dd, 1H, $J_{3eq',4'}$=4.7, $J_{3eq',3ax'}$= 12.4 Hz, H-3′$_{eq}$), 2.03 (s, 3H, C(O)C$H_3$), 1.80 (dd, 1H, $J_{3ax',4'}$=12.2 Hz, H-3′$_{ax}$), 1.67–1.58 (m, 4H, CH$_2$C$H_2$N$_3$, OCH$_2$C$H_2$), 1.43–1.37 (m, 4H, CH$_2$CH$_2$); $^{13}$C NMR (125 MHz, $D_2O$): $\delta$ = 175.9 (2C), 103.4, 100.7, 76.8, 75.7, 73.7, 72.6, 71.2, 70.0, 69.2, 69.0, 68.4, 63.5, 61.8, 52.6, 52.0, 40.6, 29.5, 28.8, 26.6, 25.5, 22.9; ESI HRMS: $m/z$: calcd for $C_{23}H_{39}N_4O_{14}$: 641.22527; found: 641.22510 $[M+Na^+]$.

**6-Aminohexyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (45)**: Azide **44** (0.024 g, 0.04 mmol) was combined with triphenylphosphine (0.053 g, 0.2 mmol, 5.0 equiv) and a mixture of pyridine and water 9:1 (5 mL) was added. The suspension was stirred at 65 °C for 36 h, then diluted with water and filtered through a plug of sand and cotton. The filtrate was concentrated and then passed through a tC18 SepPak cartridge (0.4 g) preconditioned with ~1% $NH_4OH$ before final purification by HPLC (Hamilton PRP-1 column, methanol/water gradient) to give **45** (0.0186 g, 0.03 mmol, 78%). $[\alpha]_D$ = −2.6 ($c$ = 0.40, $H_2O$); $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.46 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.08 (dd, 1H, $J_{3,2}$=9.7, $J_{3,4}$= 3.2 Hz, H-3), 3.95 (d, 1H, $J_{3,4}$=3.2 Hz, H-4), 3.93 (ddd, 1H, $J$=$J$=3.1, 9.8 Hz, OC$H_2$), 3.90–3.86 (m, 2H, H-8′, H-9a′), 3.84 (dd, 1H, $J_{5',6'}$=$J_{5',6'}$= 10.2 Hz, H-5′), 3.77–3.62 (m, 7H, H-6a, H-6b, H-4′, H-5, OC$H_2$, H-6′, H-9b′), 3.60 (dd, 1H, $J$=1.7, 9.1 Hz, H-7′), 3.54 (dd, 1H, H-2), 3.00 (dd, 2H,


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$CH_2NH_2$), 2.76 (dd, 1H, $J_{3eq',4'}$=4.8, $J_{3eq',3ax'}$=12.5 Hz, H-3'$_{eq}$), 2.03 (s, 3H, C(O)$CH_3$), 1.80 (dd, 1H, $J_{3ax,4'}$=12.0 Hz, H-3'$_{ax}$), 1.70–1.61 (m, 4H, $CH_2CH_2NH_2$, $OCH_2CH_2$), 1.45–1.39 (m, 4H, $CH_2CH_2$); $^{13}$C NMR (125 MHz, $D_2O$): $\delta$ = 175.9, 174.7, 103.4, 100.7, 76.8, 75.8, 73.8, 72.7, 71.1, 70.0, 69.2, 69.0, 68.4, 63.5, 61.9, 52.6, 52.0, 40.6, 40.3, 29.3, 27.6, 26.1, 25.4, 22.9; ESI HRMS: *m/z*: calcd for $C_{23}H_{42}N_2O_{14}$: 593.2528; found: 593.2537 [$M$+Na$^+$].

**Allyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (46)**: Allyl β-D-galactopyranoside (0.020 g, 0.09 mmol) was combined with crude CMP-Neu5Ac (crude mass 290 mg, estimate 0.10 mmol, 1.1 equiv). α(2,3) Sialyltransferase (50 mM HEPES, pH 7.5, 10% glycerol (v/v); 0.4 U mL$^{-1}$; 1.353 mL), buffer (50 mM HEPES, pH 7.5, 10% glycerol (v/v); 1.5 mL) $MnCl_2$ and $MgCl_2$ (60 μL of a 0.5 M solution; final conc. 10 mM), dithiothreitol (38 μL, 0.4 M; final conc. 3 mM) and alkaline phosphatase (3 μL; 3U) were added and the mixture was tumbled overnight. After centrifugation (30 min at 14000 rpm) the supernatant was loaded onto a Biogel P2 column (2.5×30 cm) and eluted with water. Final purification by reverse phase HPLC (Beckman C18 column, water-methanol gradient) afforded **46** (0.0311 g, 0.06 mmol, 67%). $[\alpha]_D$ = 0 ($c$ = 0.40, $H_2O$); $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.98 (dddd, 1H, $J$=$J$=5.9, 10.6, ~17 Hz, H-b), 5.37 (br d, 1H, $J$=17 Hz, H-c), 5.26 (br d, 1H, $J$=10.6 Hz, H-d), 4.50 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.38 (dd, 1H, $J$=5.7, 12.6 Hz, H-a), 4.21 (dd, 1H, $J$=6.5, 12.6 Hz, H-a'), 4.07 (dd, 1H, $J_{3,2}$=9.8, $J_{3,4}$=3.1 Hz, H-3), 3.93 (d, 1H, $J_{3,4}$=3.1 Hz, H-4), 3.88–3.83 (m, 2H, H-8', H-9a'), 3.82 (dd, 1H, $J_{5',6'}$=$J_{5',6'}$=10.2 Hz, H-5'), 3.77–3.71 (m, 2H, H-6a, H-6b), 3.70–3.57 (m, 5H, H-4', H-5, H-6', H-9b', H-7'), 3.55 (dd, 1H, H-2), 2.74 (dd, 1H, $J_{3eq',4'}$=4.5, $J_{3eq',3ax'}$=12.4 Hz, H-3'$_{eq}$), 2.02 (s, 3H, C(O)$CH_3$), 1.78 (dd, 1H, $J_{3ax',4'}$=12.0 Hz, H-3'$_{eq}$); $^{13}$C NMR (125 MHz, $D_2O$): $\delta$ = 175.9, 174.8, 134.3. 119.7, 102.5, 100.7, 76.8, 75.8, 73.7, 72.7, 71.4, 70.0, 69.2, 69.0, 68.4, 63.5, 61.8, 52.6, 40.5, 22.9; ESI HRMS: *m/z*: calcd for $C_{20}H_{32}NO_{14}$: 510.1817; found: 510.1812 [$M$+Na$^+$].

**Allyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-2-*O*-benzoyl-3-thio-β-D-galactopyranoside (47)**: Disaccharide **19** (0.285 g, 0.32 mmol) was dissolved in 80% acetic acid (10 mL) and the mixture was heated at 55 °C overnight with stirring. After evaporation of the volatiles, the residue was concentrated from toluene several times. Chromatography of the residue on silica gel by using dichloromethane/methanol (96:4) as eluent yielded **47** (0.217 g, 0.27 mmol, 85%). $[\alpha]_D$ = 22 ($c$ = 1.15, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.15–8.11 (m, 2H, Ar), 7.59–7.54 (m, 1H, Ar), 7.49–7.43 (m, 2H, Ar), 5.84–5.73 (m, 1H, H-b), 5.58 (ddd, 1H, $J$=2.8, 5.7, $J_{8',7'}$=9.8 Hz, H-8'), 5.24–5.16 (m, 3H, H-2, H-7', H-c), 5.10 (br d, 1H, N$H$), 5.05 (ddd, 1H, $J$=1.4, 1.4, 1.4, 10.5 Hz, H-d), 4.97 (d, 1H, $J$=7.7 Hz, H-1), 4.80 (ddd, 1H, $J_{4',3'eq}$=4.6, $J_{4',3'ax}$=11.7, $J_{4',5'}$=10.3 Hz, H-4'), 4.37–4.29 (m, 2H, H-9'a, H-a), 4.15 (dddd, 1H, $J$=1.4, 1.4, 5.9, 13.2 Hz, H-a'), 4.06 (dd, 1H, $J_{9'b,8'}$=5.8, $J_{9'b,9'a}$=12.5 Hz, H-9'b), 3.95–3.83 (m, 4H, H-6a, H-6b, H-5, H-5'), 3.79–3.74 (m, 1H, H-6'), 3.77 (s, 3H, $CO_2CH_3$), 3.71 (dd, 1H, $J_{3,4}$=2.7, $J_{3,2}$=11.7 Hz, H-3), 3.62 (br d, 1H, H-4), 2.60 (dd, 1H, $J_{3eq,3ax}$=12.8 Hz, H-3'$_{eq}$), 2.4–2.2 (b, 2H, 2×OH), 2.19, 2.07, 1.98, (3 s, 3×3H, 3×C(O)$CH_3$), 1.94 (dd, 1H, H-3$_{ax}$), 1.83, 1.70 (2 s, 2×3H, 2×C(O)$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 170.9, 170.8, 170.25, 170.20, 170.12, 169.4, 165.8, 133.9, 132.9, 130.3, 130.1, 128.3, 117.1, 101.4, 81.4, 75.2, 73.6, 70.3, 70.2, 69.3, 69.1, 67.4, 66.8, 63.2, 62.4, 53.2, 49.1, 48.2, 37.9, 23.1, 21.4, 20.8 (2C), 20.5; ESI HRMS: *m/z*: calcd for $C_{43}H_{51}NO_{18}SNa$: *m/z*: 836.2406; found: 836.2402 [$M$+Na$^+$]; elemental analysis calcd (%) for $C_{36}H_{47}NO_{18}S$: C 53.13, H 5.82, N 1.72; found: C 52.95, H 5.99, N 1.66.

**Allyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-3-thio-β-D-galactopyranoside (49)**: Allyl glycoside **19** (0.102 g, 0.11 mmol) was dissolved in 80% acetic acid (10 mL) and heated overnight at 45 °C. After concentrating, the residue was evaporated from toluene (3×15 mL) and suspended in dry methanol (5 mL). A small piece of sodium was added, and the mixture was stirred at room temperature for 36 h (higher temperatures resulted in loss of the N-acetamido function). After addition of several drops of water, the reaction temperature was increased to 40 °C and stirring was continued overnight. Neutralization with acetic acid followed by concentration afforded a solid which was loaded onto a SepPak cartridge (5 g) in a minimum volume of water. Elution with water afforded **49** (0.049 g, 0.09 mmol, 81%). $[\alpha]_D$ = 50 ($c$ = 0.68, MeOH); $^1$H NMR (500 MHz, $D_2O$): $\delta$ = 6.04–5.96 (m, 1H, H-b), 5.42–5.37 (m, 1H, H-c), 5.30–5.27 (m, 1H, H-d), 4.53 (d, 1H, $J_{1,2}$=7.3 Hz, H-1), 4.43–4.39 (m, 1H, H-a), 4.26–4.22 (m, 1H, H-a'), 3.93–3.82 (m, 4H, H-8', H-9a', H-4, H-5'), 3.75–3.71 (m, 3H, H-5, H-6a, H-6b), 3.70–3.62 (m, 2H, H-4', H-9b'), 3.60–3.56 (m, 2H, H-6', H-7'), 3.39 (dd, 1H, $J_{2,3}$=11.4 Hz, H-2), 3.35 (dd, 1H, $J_{3,4}$=2.7 Hz, H-3), 2.81 (dd, 1H, $J_{3eq,4}$=4.8, $J_{3eq,3ax}$=12.7 Hz, H-3'$_{eq}$), 2.03 (s, 3H, NHC(O)$CH_3$), 1.82 (dd, 1H, $J_{3ax,4}$=11.7 Hz, H-3'$_{ax}$); $^{13}$C NMR (125 MHz, $D_2O$): $\delta$ = 175.9, 175.4, 134.3, 119.6, 104.0, 85.1, 78.3, 75.8, 72.8, 71.4, 69.7, 69.4, 69.1, 69.0, 63.4, 62.1, 52.5, 51.8, 41.6, 22.9; ESI HRMS: *m/z*: calcd for $C_{20}H_{32}NO_{13}S$: 526.1589; found: 526.1585 [$M$+Na$^+$].

**Preparation of TT and BSA conjugates**

**Procedure for preparation of TT conjugates 36 and 37 with adipate linker**: Purified tetanus toxoid (~6.6 mg mL$^{-1}$) in PBS, pH 7.2, was added to the activated oligosaccharide (**34** or **35**) (~30 mol equiv). The mixture was tumbled gently overnight before being dialyzed against PBS (5×2 L) at 4 °C.

**Procedure for preparation of BSA conjugates 38 and 39 with adipate linker**: Bovine serum albumin (Sigma) (~3.5 mg mL$^{-1}$) in PBS, pH 7.2, was added to the adipate activated oligosaccharide (**34** or **35**) (~30 mol equiv). The mixture was tumbled gently overnight, dialyzed against milliQ water (5×2 L) at 4 °C, and then lyophilized. The glycoconjugate was stored at −20 °C prior to use.

**Procedure for preparation of BSA conjugates with diethyl squarate**: Sugar amines (**1**, **2**, **40**–**43** and **45**) were dissolved in methanol and 3,4-diethoxy-3-cyclobutene-1,2-dione (0.9 equiv) was added in methanol. Reaction progress was monitored by TLC, and when necessary, sodium methoxide was added. When all starting material had been consumed as judged by TLC, the mixture was transferred to an Epindorph tube and solvent was removed with a stream of argon. The residue was dried in vacuo for 1 h, after which time a solution of BSA (5 mg mL$^{-1}$) in borate buffer, pH 9.0 was added. The solutions were tumbled overnight and the conjugates processed and stored as described for **38** and **39**.

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