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Synthesis of ganglioside epitopes for oligosaccharide specific immunoadsorption therapy of Guillian-Barré syndrome †

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Guillain-Barré syndrome is a postinfectious, autoimmune neuropathy resulting in neuromuscular paralysis. Auto-antibodies, often induced by bacterial infection, bind to human gangliosides possessing monosialoside and diasialoside epitopes and impair the function of nerve junctions, where these ganglioside structures are highly enriched. Truncated gangliosides representive of GD_3, GQ_{1b} and GM_2 epitopes have been synthesized as methyl glycosides and as a glycosides of an eleven carbon tether. The synthetic oligosaccharide ligands are structural mimics of these highly complex ganglioside epitopes and *via* their ability to neutralize or remove auto-antibodies have the potential for therapy, either as soluble blocking ligands administered systemically, or as immuno-affinity ligands for use as extracorporeal immunoadsorbents.

Introduction

"Molecular mimicry" or cross immune reaction initiated by *Campylobacter jejuni* LPS that possess human glycolipid like structure is inferred to provide the antigenic stimulus for an anti-ganglioside auto-antibody response in patients, who develop neurological paralysis.[1–6] An approach is described that utilizes synthetic oligosaccharide ligands to neutralize or remove these auto-antibodies.

A wide variety of acute and chronic peripheral neuropathies, which have an autoimmune basis, are associated with circulating antibodies directed to ganglioside and other glycolipid antigens.[7–16] Guillain-Barré syndrome (GBS), the foremost cause of neuromuscular paralysis, is a postinfectious, autoimmune neuropathy that frequently follows *Campylobacter jejuni* enteritis. GBS is particularly associated with different patterns of anti-ganglioside antibodies which segregate according to the clinical phenotype.[7,17–20] The clinical features of GBS and its clinical variant Miller-Fisher syndrome (MFS) may co-occur in any given patient to produce an overlap syndrome.[19] More than 90% of MFS cases and GBS overlap cases have acute-phase antibodies to GQ_{1b} and GT_{1a} gangliosides that disappear with clinical recovery.[7,9] MFS patient sera may also contain antibodies that react with structurally similar gangliosides containing disialosyl residues, including GD_3, GD_{1b} and GT_{1b}[10,16] (Table 1). Of special interest are the ganglioside antigens that express the disialoside epitope, αNeuNAc(2⟶8)α-NeuNAc,[9,10,13,16] since anti-ganglioside antibody-reactive disialoside epitopes are widely distributed in peripheral nerve, and antibodies to these antigens cause clinical, electrophysiological and immunopathological abnormalities in a variety of model systems.[21–24]

Structural and serological studies have shown that the LPS core oligosaccharides of *C. jejuni* isolated from GBS and MFS cases share oligosaccharide sequences that are identical with the terminal segments of gangliosides, and in this sense can mimic gangliosides.[25] It has also been demonstrated that *C. jejuni* LPS is a more potent antigen in eliciting anti-ganglioside antibodies than ganglioside antigens.[26] As well, the outer core oligosaccharides of LPS[12,25,27–30] from some *Campylobacter jejuni* strains are virtually homologous with gangliosides that present the disialoside epitope (Table 2).[1,28,30] Together these observations suggest that *C. jejuni* cell wall LPS is a potent antigen capable of inducing ganglioside specific antibodies.

Ganglioside auto-antibodies are potentially hazardous because these glycolipids are highly enriched in distinct regional patterns within the nervous system; in particular, the ganglioside with two disialoside epitopes, GQ_{1b} is concentrated in extraocular nerves, the principal motor site affected in MFS.[31] Consequently, it is assumed that antibodies induce neuropathy by binding to nerve surface gangliosides and activating pro-inflammatory pathways or by causing physiological block of ganglioside-modulated peripheral nerve functions.[7,22,23,32,33]

Onset of GBS can be rapid, leading to total paralysis within 48 hours, or can evolve over several weeks. All age groups are affected, with a peak in young adults. The therapeutic window for GBS is short, currently estimated at <14 days from onset. Optimal treatment with intravenous immunoglobulin or plasma exchange only reduces illness severity by half.[21–23] The patients left severely disabled (~12% of survivors are unable to walk after 1 year) or dead (mortality of 3–10%) represent a major social and economic burden, both in the acute phase (~30% require intensive care/mechanical ventilation) and long term. Thus there is a pressing need to understand GBS pathogenesis as a prerequisite to developing effective, targeted immunotherapy.

Willison's group have proposed that anti-ganglioside antibody reactive epitopes present on nerve cells directly contribute to neuropathy pathogenesis.[34,35] Consequently oligosaccharide inhibitors or structural mimics of these complex carbohydrate epitopes have the potential for therapy, either as soluble blocking ligands administered systemically, or as immuno-affinity ligands employed as extracorporeal immunoadsorbents. To substantiate this hypothesis and the feasibility of the approach we have synthesized truncated ganglioside epitopes as methyl glycosides and as glycosides of an eleven carbon tether

† This paper is dedicated to the memory of Professor Christian Pedersen.

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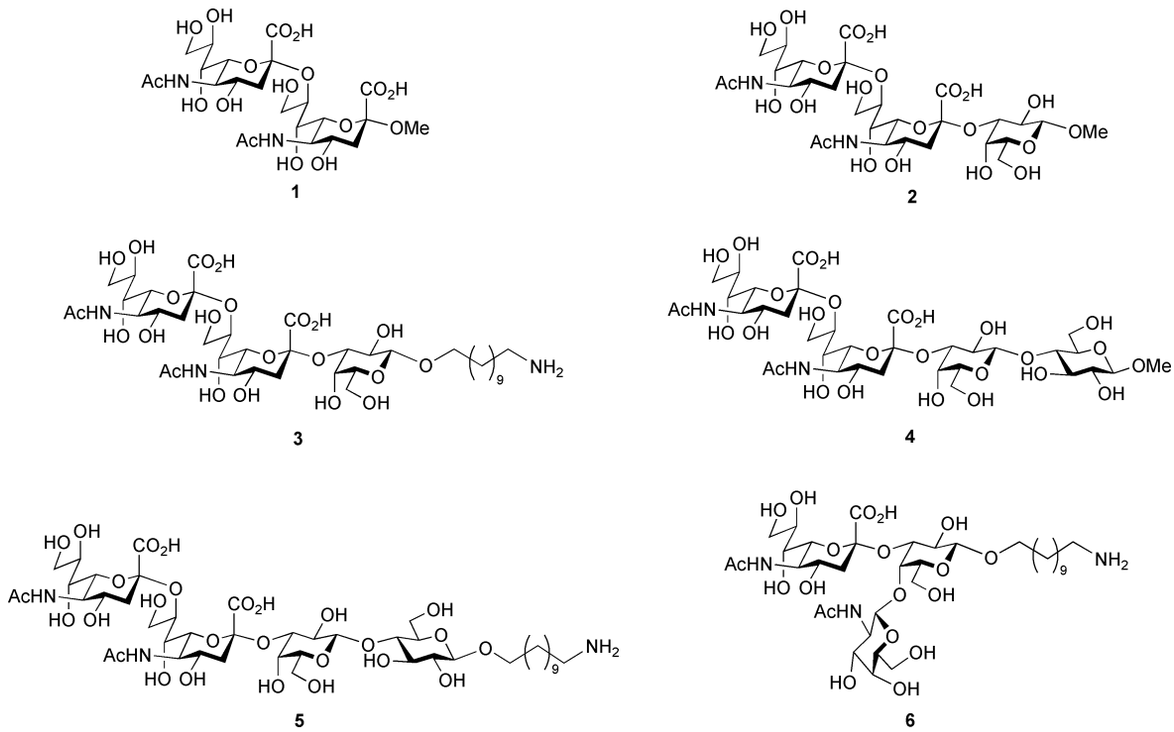
Table 1 Ganglioside nomenclature and oligosaccharide structures

Ganglioside	Oligosaccharide structure
GM_1	Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc(β1-ceramide) | NeuNAc(α2-3)
GD_3	NeuNAc(α2-8)NeuNAc(α2-3)Gal(β1-4)Glc(β1-ceramide)
GD_2	GalNAc(β1-4)Gal(β1-4)Glc(β1-ceramide) | NeuNAc(α2-8)NeuNAc(α2-3)
GD_{1a}	NeuNAc(α2-3)Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc(β1-ceramide) | NeuNAc(α2-3)
GD_{1b}	Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc(β1-ceramide) | NeuNAc(α2-8)NeuNAc(α2-3)
GT_{1a}	NeuNAc(α2-8)NeuNAc(α2-3)Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc(β1-ceramide) | NeuNAc(α2-3)
GT_{1b}	NeuNAc(α2-3)Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc(β1-ceramide) | NeuNAc(α2-8)NeuNAc(α2-3)
GQ_{1b}	NeuNAc(α2-8)NeuNAc(α2-3)Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc(β1-ceramide) | NeuNAc(α2-8)NeuNAc(α2-3)

Table 2 Outer core structures of *C. jejuni* LPS that correlate with ganglioside epitopes

Bacterial serostrain	*C. jejuni* LPS outer core structure
O:4 and O:19	Gal(β1-3)GalNAc(β1-4)Gal(β1-3)Hep(α1-3)Hep | NeuNAc(α2-3)
O:4 and O:19	NeuNAc(α2-3)Gal(β1-3)GalNAc(β1-4)Gal(β1-3)Hep(α1-3)Hep | NeuNAc(α2-3)
O:19 OH4384	NeuNAc(α2-8)NeuNAc(α2-3)Gal(β1-3)GalNAc(β1-4)Gal(β1-3)Hep(α1-3)Hep | NeuNAc(α2-3)
O:19 OH4382	NeuNAc(α2-8)NeuNAc(α2-3)Gal(β1-3)Hep(α1-3)Hep

containing the αNeuNAc(2⟶8)αNeuNAc disaccharide itself and this same disialosyl unit attached to larger structures **2**–**6** (Scheme 1). The tethered derivatives were used to prepare a variety of glycoconjugates for use as ELISA screening antigens with an extensive panel of clinically relevant polyclonal and monoclonal antibodies and to establish the epitope size by inhibition with soluble oligosaccharides. The tethered oligosaccharides were incorporated into extracorporeal immunoadsorbents to test the principle of removing peripheral nerve specific anti-ganglioside antibodies from patient plasma.



Scheme 1

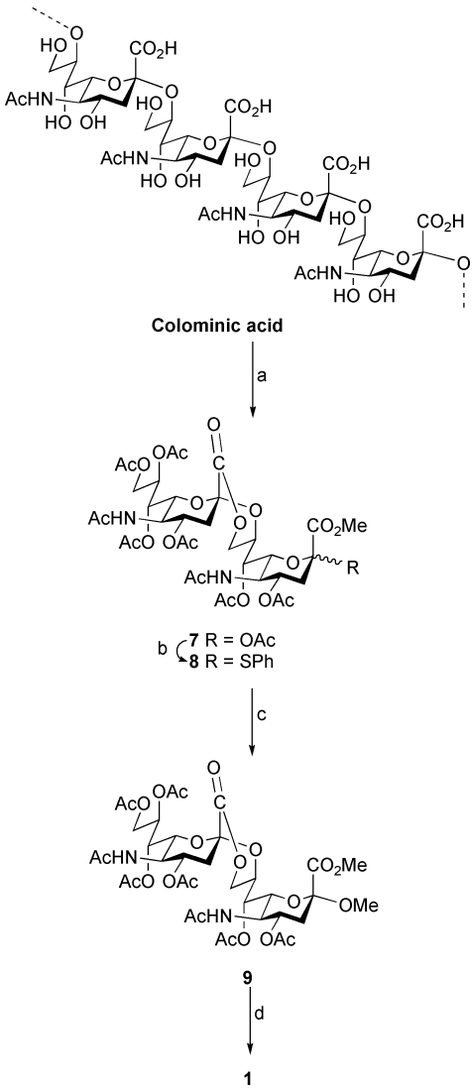
Results

The pivotal decision in the synthetic approach to the target epitopes **1**–**5** was the use of the disialoside donor **8**. A related disaccharide synthon was previously synthesized following controlled acid hydrolysis of α2,8-poly-*N*-acetylneuraminic acid (colominic acid sodium salt).[36] Here we adopt this general strategy but report a more concise and convenient preparative route to the disialoside lactone **7** (Scheme 2). In the procedure reported by Roy and Pon,[36] hydrolysis of colominic acid was performed on a 100 mg scale at pH 3.0, 70 °C for 3 h. The hydrolysed mixture was then lyophilised and subjected to gel permeation chromatography on a Bio-Gel P-10 column to afford the disaccharide as the main product in a yield of 28%. Although the chromatographic step allows the successful separation of disialoside from sialic acid and higher oligomers, it is time consuming and imposes a significant limiting factor on the scale of the reaction. When the hydrolysis of colominic acid was performed under the slightly milder conditions (pH 3.8) and 70 °C, the formation of the disaccharide was consistently reproducible. In addition, the gel filtration step was eliminated and instead the crude hydrolysate was subjected to esterification/lactonization and per-acetylation steps without isolating the intermediate reaction products. In fact, by omitting the gel filtration step, the transformation of colominic acid to the per-acetylated lactone **7** was consistently achieved on a 5–10 gram scale, with the improved overall yield (>33%, over 4 steps). Only a single chromatography step on silica gel was necessary. This improved approach incorporated an additional modification of the procedure to esterify and lactonize the two carboxylic acid residues, which previously required stirring the disialoside for two days with Amberlite IR-120 (H^+) resin at 40 °C.[37] In our hands this procedure was sensitive to moisture and difficult to reproduce since the disialoside linkage tends to hydrolyse. However, brief treatment of the crude hydrolysate with a methanolic suspension of acidic ion exchange resin (10 min) and co-concentration with a methanolic solution of hydrochloric acid effected complete esterification and lactonization within one hour. Compound **7** was then converted into phenyl thioglycoside **8** in 88% yield as previously reported.[37] Reaction of this donor with methanol gave the methyl disialoside **9** in moderate yield (33%) followed by opening of the lactone and de-acetylation to give the disialic acid methyl glycoside **1** (80%).

In order to prepare analogues and terminal elements of gangliosides that possess disialoside containing epitopes (*e.g.* GD_3, GQ_{1b} *etc.*) as well as the branched element of gangliosides such as GM_2, GT_{1a} partially protected galactose or lactose derivatives **10**, **18**, **19** and **25** were employed (Scheme 3). The galactoside **10** and **19** were synthesized according to literature procedures.[38,39] The tether galactoside **18** was synthesized using the ω-azido alcohol **12**, prepared by substitution of 11-bromo-undecanol **11** in excellent yield (96%). Glycosylation of **12** with the known tetrabenzoylgalactosyl bromide **13**[40] promoted by silver triflate in toluene afforded the intermediate glycoside **14**, which was not isolated, but deprotected to afford the tetraol **15** in 57% overall yield from D-galactose. After installation of the 3,4-*O*-isopropylidene acetal (⟶ **16**, 96%), diol **16** was perbenzylated in excellent yield (⟶ **17**, 90%). Removal of the isopropylidene group by acid hydrolysis afforded the desired acceptor **18** (95%). In an analogous fashion, the known perbenzoylated lactosyl bromide **20**[41] was glycosidated with alcohol **12** under silver triflate activation in toluene to afford the intermediate glycoside **21**. Again, **21** was not isolated, but directly deprotected to afford **22** in 73% overall yield from D-lactose. The 3′,4′-positions were selectively protected as the isopropylidene acetal (⟶ **23**, 87%), and the pentol **23** was perbenzylated (⟶ **24**, 92%). After removal of the isopropylidene group by acid hydrolysis, the desired acceptor **25** was obtained in excellent yield (96%).

The trisaccharide methyl glycoside **2** was prepared according to Scheme 4. Thus, glycosylation of **10** with **8** in dry acetonitrile at −30 °C for three days in the presence of *N*-iodosuccinimide (NIS) and triflic acid afforded the α-glycoside **26** which was difficult to purify. However, when all the ester linkages in **26** were saponified, the dibenzylated trisaccharide **27** could be easily isolated by HPLC in 28% overall yield from **8**. Hydrogenation of **27** afforded the desired methyl glycoside **2** in excellent yield (91%).

The tethered trisaccharide analogue **3** was prepared in a similar fashion to **26** (Scheme 5). Coupling of **18** with **8** under the same conditions as **26** afforded the intermediate α-glycoside



Scheme 2 *Reagents and conditions*: a. 1) H_2O, pH = 3.8, 70 °C; 2) MeOH/Dowex (H^+), then HCl/MeOH; 3) Ac_2O/Py; b. PhSH, BF_3·Et_2O/CH_2Cl_2; c. MeOH, NIS, TfOH/CH_3CN, −35 °C; d. NaOMe/MeOH; then H_2O.

28. As before this trisaccharide was not subjected to extensive purification, but was partially deprotected and the dibenzylated compound **29** was obtained in pure form after purification by HPLC (22% overall from **8**). Dissolving metal deprotection at low temperature (−78 °C) successfully removed both the benzyl groups, and simultaneously reduced the azido group to the amine to yield the glycoside **3** in quantitative yield (99%).

The GD_3 methyl glycoside **4** was prepared by coupling the acceptor **19** with the disialyl donor **8**, in the presence of *N*-iodosuccinimide–triflic acid (Scheme 6) to afford tetrasaccharide **30**, which could only be isolated in pure form and moderate yield (25%) after HPLC chromatography on silica gel column. The tetrasaccharide **4** was finally obtained after saponifying the ester linkages and hydrogenolysis of benzyl groups (⟶ **4**, 89%).

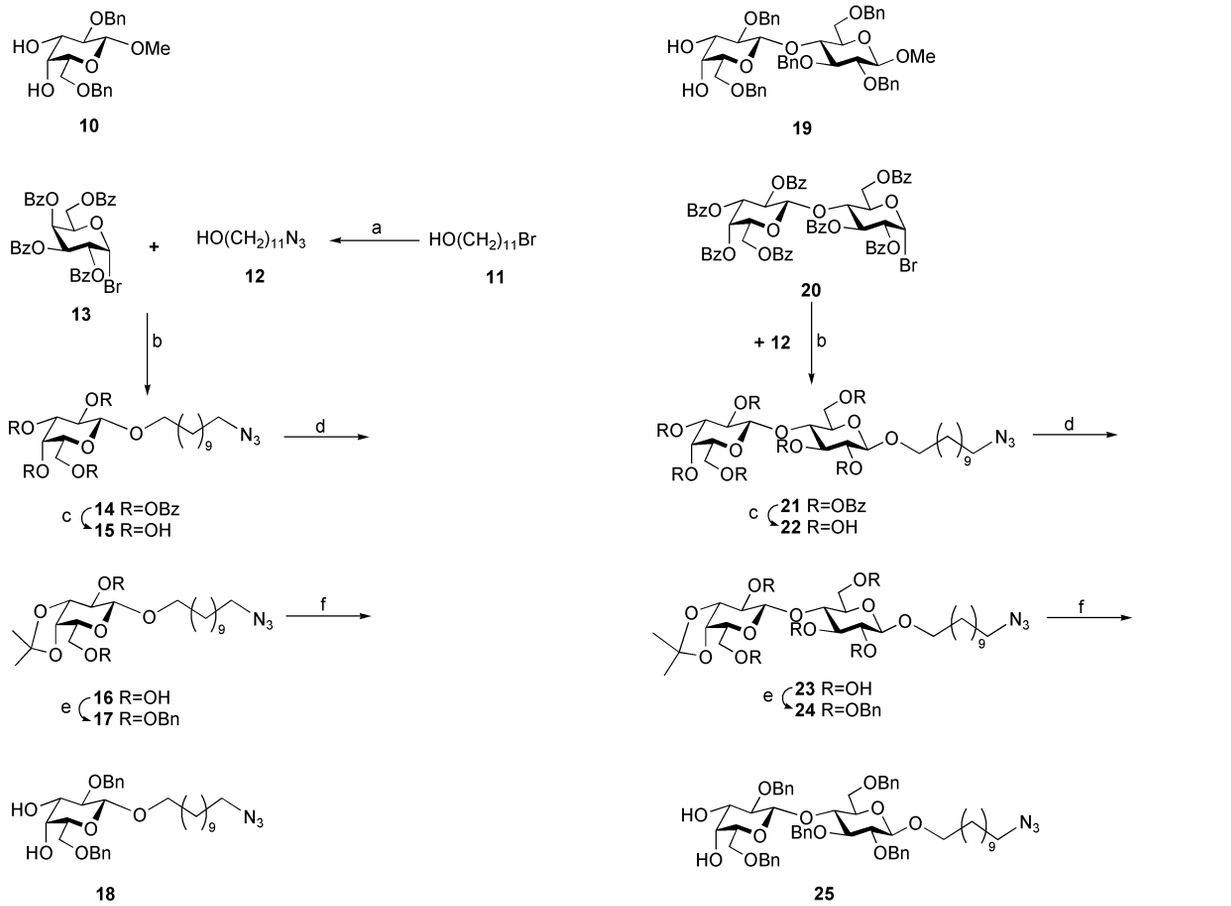
The tether GD_3 analogue **5** was prepared by coupling the diol **25** with the disialyl donor **8** under the same activation conditions as **30** (Scheme 7) to afford the tetrasaccharide **31**, which was purified by HPLC chromatography on a silica gel column (29%). The tetrasaccharide **5** was obtained after saponifying the ester linkages, and removing the benzyl groups by dissolving metal conditions, which achieved the concomitant reduction of the azido group to the amine (⟶ **5**, 73%).

The branched GM_2 type trisaccharide **6** was prepared using acceptor **18** (Scheme 8). Sialylation of **18** with the *N*,*N*-diacetyl sialyl donor **32**[42] in a 2 : 1 mixture of acetonitrile–propionitrile at −65 °C afforded the desired disaccharide **33** in 46% yield under the same conditions as above. In order to prepare the desired trisaccharide, a galactosamine donor was required. We chose to use the 2-deoxy-2-phthalimido thiogalactoside **36** as the preferred donor, because it possesses a 3,4-*O*-isopropylidene acetal, which should be an interesting intermediate for the synthesis of more complex oligosaccharides. Compound **36** was prepared from the known triol **34**[43] by selectively protecting the 3,4 *cis*-diol using an isopropylidene group (⟶ **35**, 86%) and subsequently benzoylating the primary alcohol (⟶ **36**, 97%). The glycosylation of disaccharide **33** with donor **36** was carried out in anhydrous acetonitrile in the presence of *N*-iodosuccinimide–triflic acid at 0 °C to give the desired trisaccharide **37** in moderate yield (48%). Removal of protection groups followed a five-step reaction sequence. The isopropylidene was first removed by mild acidic hydrolysis using 90% aqueous acetic acid at 70 °C; the acetyl protecting groups were removed by Zémplen transesterification and the methyl ester of sialic acid was saponified by adding water to the basic methanol solution. In order to convert the phthalimido to acetamido group, the mixture was treated with hydrazine in *n*-butanol at elevated temperature to yield the intermediate free amine which was acetylated by acetic anhydride in methanol. The benzyl groups were removed and the azido group, reduced using dissolving metal deprotection conditions to afford the desired trisaccharide **6**, which was purifed by reversed phase chromatography on a C18 column (34% overall).

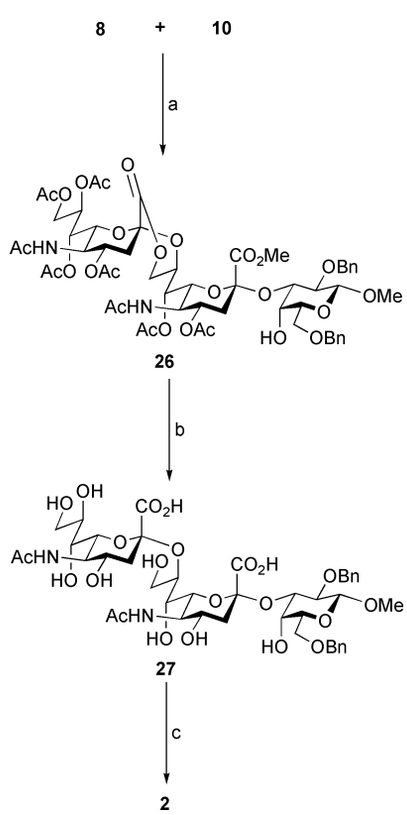
Affinity columns were prepared from the tethered glycosides using commercially available *N*-hydroxysuccinimide (NHS) activated sepharose gel (Scheme 9). The tether functionalized ligands **3**, **6** were reacted with the gel in buffer at pH 7 and subsequently the remaining active sites were blocked by reaction with excess ethanolamine. Unreacted trisaccharides **3** and **6** were recovered by filtration through C18-SepPak cartridges or HPLC chromatography. Based on recovered ligands the coupling efficiencies were 65% for **3** and 54% for **6**. This resulted in affinity columns with a density of ~0.6 μmol oligosaccharide/ml dry gel in both cases.

Coupling of compounds **3**, **5** and **6** to bovine serum albumin (BSA) protein in order to create glycoconjugates for use in enzyme immunoadsorbent assays (ELISA) was accomplished through a squarate linker.[44] Diethyl squarate, 3,4-diethoxy-3-cyclobutene-1,2-dione, conveniently permits reaction with the tether amino group in methanol or aqueous sodium carbonate solution to afford the corresponding squarate half esters, which were subsequently coupled to BSA protein through the terminal amine group of exposed lysine residues of the protein. When a ligand/protein ratio of 10–12/1 was employed, the average number of ligands per BSA ranging from 6–8.4 as determined by MALDI-TOF-MS (for conjugation of **3** and **5**). When this ratio was increased to 20/1, the average number of ligands per BSA increased to 14 (for conjugation **6**).

The glyconconjugates and affinity columns have been evaluated for their ability to bind to antibodies present in the serum of patients with GBS and MFS.[45] An example of the effectiveness of the BSA glycoconjugate prepared from **3** in binding a GD_3 monoclonal antibody (R24)[46] is shown Fig. 1. The glycoconjugate is as good or better than the ganglioside GD_3 in binding R24 antibody. The same disialylgalactose glycoconjugate synthesized from **3** binds anti-GQ1b antibodies in 32/58 (55%) human sera containing IgG or IgM anti-GQ1b antibodies at titres up to 1/130000, and also binds a wide range of mouse monoclonal anti-GQ1b and -GD3 antibodies. When conjugated to Sepharose as mock therapeutic immmuno-affinity columns, the immobilized trisaccharide (**3**-Sepharose) eliminates anti-GQ1b antibodies from positive sera in proportion to their level of binding to DSG-BSA.[44] Oligosaccharide-specific immunoadsorption therapy may provide a new therapeutic approach to anti-ganglioside antibody-associated GBS.



Scheme 3 *Reagents and conditions*: a. NaN_3/DMF, 110⟶130 °C; b. AgOTf/C_6H_5Me, −78⟶10 °C; c. NaOMe/MeOH, reflux; d. 2,2-dimethoxypropane/CSA, reflux; e. BnBr, NaH/DMF; f. AcOH/H_2O, 85 °C.



Scheme 4 *Reagents and conditions*: a. NIS, TfOH/CH_3CN, −30 °C; b. NaOMe/MeOH, then H_2O; c. H_2, $Pd(OH)_2$-C/MeOH.

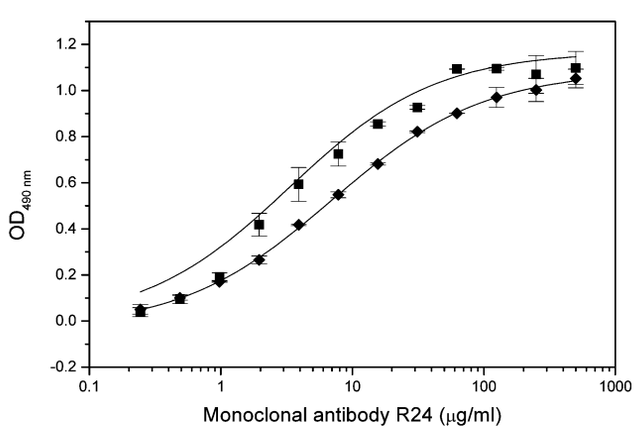
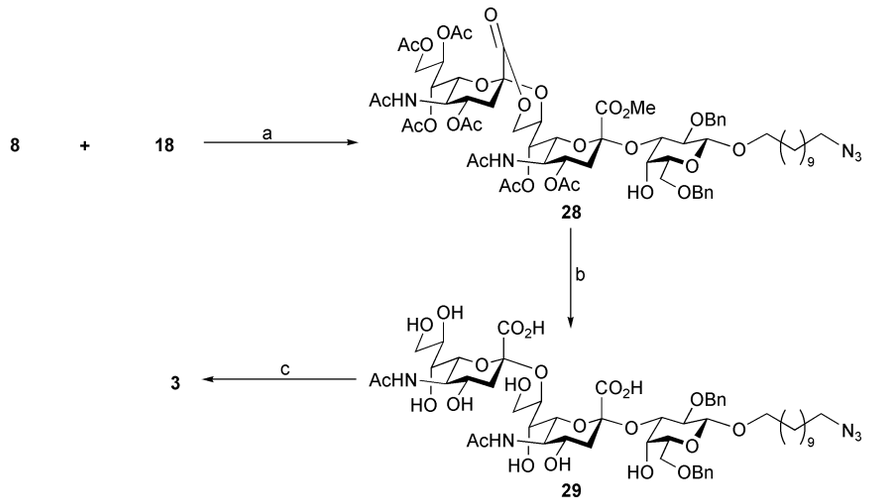


Fig. 1 ELISA titration curves for the monoclonal anti-GD_3 antibody R24 against two antigens coated on ELISA plates; (i) coglyconjugate produced by ligand **3** conjugated to BSA (■) and (ii) GD_3 glycolipid (◆).

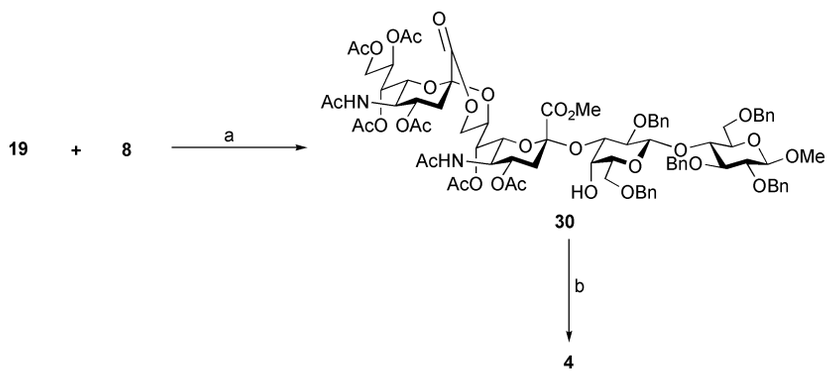
Experimental

General methods

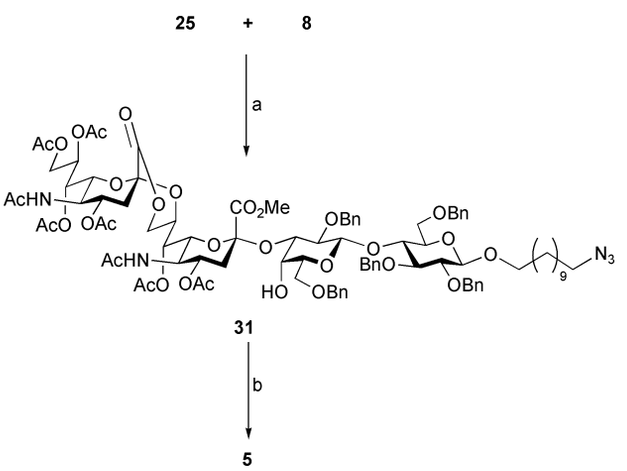
The optical rotations were measured with a Perkin-Elmer 241 polarimeter for samples in a 10 cm cell at 22 ± 2 °C. $[a]_D$ values are given in units of 10^{-1} deg cm² g^{-1}. Analytical TLC was performed on silica gel 60-F_{254} (Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 5% sulfuric acid in water. All commercial reagents were used as supplied and chromatography solvents were distilled prior to use. Column chromatography was performed on silica gel 60 (Silicycle, Ontario). ^{1}H NMR spectra were recorded on Varian Unity 300, 500, or 600 MHz spectrometers. First order proton



Scheme 5 *Reagents and conditions*: a. NIS, TfOH/CH_3CN, −30 °C; b. NaOMe/MeOH, then H_2O; c. Na/NH_3, −78 °C.



Scheme 6 *Reagents and conditions*: a. NIS, TfOH/CH_3CN, −30 °C; b. (1) NaOMe/MeOH, then H_2O; (2) H_2, $Pd(OH)_2$-C/MeOH.

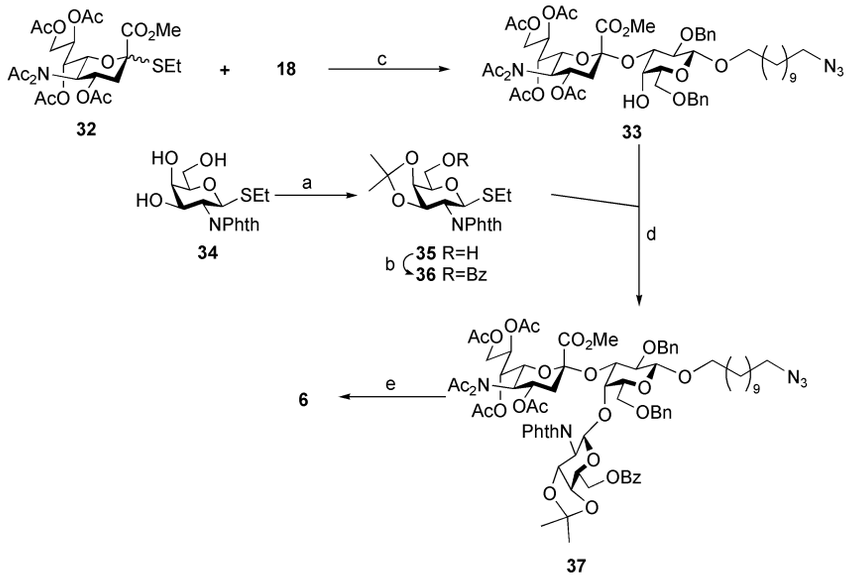


Scheme 7 *Reagents and conditions*: a. NIS, TfOH/CH_3CN, −30 °C; b. (1) NaOMe/MeOH, then H_2O; (2) Na/NH_3, −78 °C.

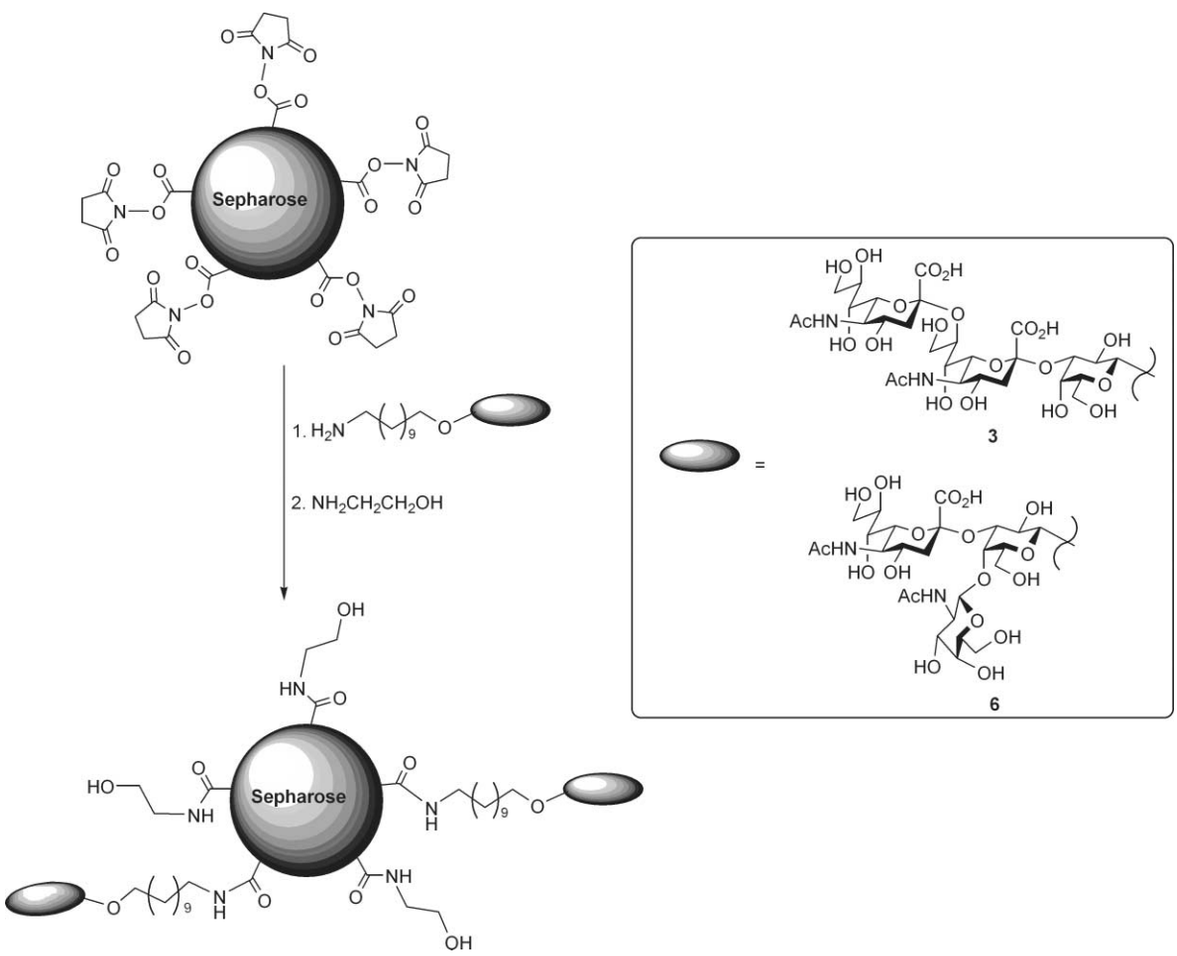
chemical shifts δ_H are referenced to either residual $CHCl_3$ (δ_H 7.24, $CDCl_3$) or CD_2HOD (δ_H 3.30, CD_3OD), or internal acetone (δ_H 2.225, D_2O). The assignment of resonances for all compounds was made by two-dimensional homonuclear and heteronuclear chemical shift correlation experiments. For clarity in reporting chemical shifts of all the disialylated compounds, letter A as suffix designates sialic acid units at the non-reducing terminus and letter B as suffix internal sialic acid units. Molecular sieves were crushed and stored in an oven at 150 °C and flame dried under vacuum before use. Organic solutions were dried with anhydrous Na_2SO_4 prior to concentration under vacuum at < 40 °C (bath). All final compounds were purified by reverse phase chromatography performed on a Waters 600 HPLC systems, using either a Waters semi-preparative C-18 column (10 × 250 mm, 5 μ) or Waters semi-preparative C-8 column (10 × 250 mm, 5 μ), and the products were detected with a Waters 2487 UV detector or a Waters 2410 refractive index monitor. Microanalyses and electro-spray mass spectra were performed by the analytical services of this department.

Methyl 5-acetamido-2,4,7-tri-*O*-acetyl-3,5-dideoxy-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1′,9-lactone)-D-*glycero*-D-*galacto*-2-nonulopyranosonate (7). Colominic acid (5.14 g) was dissolved in water (100 ml) and the pH of the solution was adjusted to 3.8 using 1 M HCl. The reaction mixture was stirred at 70 °C and the progress of the reaction was monitored by TLC (isopropanol–water–aqueous ammonia (~30%), 7 : 2 : 1). After 3 h the solution was cooled to −78 °C and freeze-dried. The residue was dissolved in dry methanol (200 ml) and the solution was treated with Dowex 50 (H^+) resin (5 ml dry) for 10 min. The resin was filtered off and the filtrate was concentrated and co-concentrated with 0.1 M hydrochloric acid in methanol (3 × 50 ml). Pyridine (50 ml) and acetic anhydride (50 ml) were added and the mixture was stirred for 24 h at room temperature. The reaction mixture was concentrated and co-evaporated with toluene (3 × 50 ml) and then the residue was purified by chromatography on silica gel (toluene–acetone 6 : 4 ⟶ 4 : 6) to give **7** (2.45 g, 33%) as an amorphous mass. Data in accordance with literature.[36]

Methyl phenyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylono-1′,9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-non-2-ulopyranosid]onate (8). To a solution of **7** (1.50 g, 1.7 mmol) and thiophenol (0.75 ml, 7.3 mmol) in dry dichloromethane (20 ml) was added $BF_3{\cdot}Et_2O$ (1.0 ml, 8.0 mmol), and the mixture was stirred for 2 days at room



Scheme 8 *Reagents and conditions*: a. 2,2-dimethoxypropane/CSA, reflux; b. BzCl/pyridine; c. NIS, $TfOH/CH_3CN/CH_3CH_2CN$, −65 °C; d. NIS, $TfOH/CH_3CN$, 0 °C; e. (1) $AcOH/H_2O$, 70 °C; (2) NaOMe/MeOH, then H_2O; (3) NH_2NH_2/*n*-BuOH, 110 °C; (4) Ac_2O/MeOH; (5) Na/NH_3, −78 °C.



Scheme 9

temperature. After diluting the mixture with dichloromethane (100 ml), the organic phase was washed with 1.0 M sodium carbonate (25 ml), sat. brine (25 ml), dried and concentrated to a syrup, which was purified by chromatography on silica gel (toluene–acetone 7 : 3 ⟶ 5 : 5) to give **8** (1.40 g, 88%) as an amorphous mass. Data in accordance with literature.[37]

Methyl[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyrano-sylano-1′,9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid]onate (9). A mixture containing compound **8** (70 mg, 0.074 mmol), anhydrous MeOH (30 µl, 0.74 mmol) and 3 Å molecular sieves (200 mg) in anhydrous CH_3CN (1.0 ml) was stirred under an atomosphere of argon at room temperature for 5 h and cooled to −35 °C. NIS (26.0 mg, 0.11 mmol) was added and a catalytic amount of triflic acid (7 µl) was added dropwise; the reaction was continued for 24 h at the same temperature. Et_3N (0.5 ml) was added to quench the reaction, and the reaction mixture was diluted with CH_2Cl_2 (50 ml), and filtered. The filtrate was washed 10% aqueous

$Na_2S_2O_3$ solution (25 ml), dried and evaporated. The residue was first purified by chromatography on silica gel (30⟶50% acetone–toluene) to afford compound **9**, which was contaminated with its β-isomer (27 mg). Pure compound **9** (21 mg, 33%) was obtained after further purification by HPLC chromatography on silica gel (0⟶2% MeOH–CH_2Cl_2). $[\alpha]_D$ −33.4° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.45 (ddd, 1H, $J_{3e,4}$ = 5.4, $J_{3a,4} \approx J_{4,5}$ = 10.6, H-4_Neu_B), 5.33 (dd, 1H, $J_{6,7}$ = 2.1, $J_{7,8}$ = 9.3, H-7_Neu_B), 5.32 (d, 1H, $J_{5,NH}$ = 9.7, NH_Neu_A), 5.29 (dd, 1H, $J_{6,7}$ = 1.7, $J_{7,8}$ = 5.7, H-7_Neu_A), 5.19 (d, 1H, $J_{5,NH}$ = 10.3, NH_Neu_B), 5.11 (ddd, 1H, $J_{8,9a}$ = 3.0, $J_{8,9b}$ = 4.8, H-8_Neu_B), 5.03 (ddd, 1H, $J_{3e,4}$ = 5.0, $J_{4,5}$ = 10.5, $J_{3a,4}$ = 11.8, H-4_Neu_A), 4.63 (‘t’, 1H, H-9a_Neu_A), 4.51 (dd, 1H, $J_{8,9b}$ = 3.7, $J_{9a,9b}$ = 12.2, H-9b_Neu_A), 4.37 (ddd, 1H, $J_{7,8}$ = 5.9, $J_{8,9a}$ = 9.5, H-8_Neu_A), 4.28 (dd, 1H, $J_{9a,9b}$ = 12.8, H-9a_Neu_B), 4.18 (d‘t’, 1H, $J_{5,6}$ = 10.6, H-5_N*eu_B), 4.09 (dd, 1H, $J_{5,6}$ = 10.6, H-6_Neu_A), 4.00 (dd, 1H, H-9b_Neu_B), 3.90 (d‘t’, 1H, H-5_Neu_A), 3.83 (s, 3H, COOMe), 3.74 (dd, 1H, H-6_Neu_B), 3.38 (s, 3H, OMe), 2.61 (dd, 1H, $J_{3a,3b}$ = 13.0, H-3e_Neu_A), 2.44 (dd, 1H, $J_{3a,3b}$ =13.4, H-3e_Neu_B), 2.19, 2.10, 2.06 (3 × s, 3 × 3H, 3 × OAc), 2.02 (s, 6H, 2 × OAc), 1.94 (dd, 1H, H-3a_Neu_A), 1.92 (dd, 1H, H-3a), 1.90, 1.87 (2 × s, 2 × 3H, 2 × OAc). High Res. ESMS *m/e* Found: M 885.27501. Calc. for $C_{36}H_{50}N_2O_{22}Na$: MNa 885.27529.

Methyl *O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid]-(2⟶8)-5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid (1). To a solution of compound **9** (19.0 mg, 0.022 mmol) in anhydrous MeOH (10 ml) was added a solution of 1.5 M MeONa in MeOH (0.1 ml), and the mixture was stirred at room temperature for 3 h. After concentration to dryness, the residue was dissolved in MeOH (5 ml), and H_2O (10 drops) was added; the mixture was stirred for 24 h at room temperature. The mixture was neutralized by addition of a piece of dry-ice, and concentrated under reduced pressure. The residue was purified by reverse phase HPLC on a C18 column using H_2O as eluent, and the fractions containing **1** were collected and lyophilised (10.8 mg, 80%). $[\alpha]_D$ +12° (*c* 0.6, H_2O). ^{1}H NMR (D_2O): δ 4.20 (m, 1H, H-8_Neu_A), 4.07 (dd, 1H, $J_{8,9a}$ = 4.0, $J_{9a,9b}$ = 12.1, H-9a_Neu_A), 3.53–3.92 (m, 12H, H-4_Neu_A + H-5_Neu_A + H-6_Neu_A + H-7_Neu_A + H-9b_Neu_A + H-4_Neu_B + H-5_Neu_B + H-6_Neu_B + H-7_Neu_B + H-8_Neu_B + H-9a_Neu_B + H-9b_Neu_B), 3.32 (s, 3H, OMe), 2.76 (dd, 1H, $J_{3e,4}$ = 4.7, $J_{3a,3e}$ = 12.5, H-3e_Neu_A), 2.62 (dd, 1H, $J_{3e,4}$ = 4.5, $J_{3a,3e}$ = 12.4, H-3e_Neu_B), 2.07, 2.03 (2 × s, 2 × 3H, 2 × Ac), 1.74 (‘t’, 1H, H-3a_Neu_A), 1.58 (‘t’, 1H, H-3a_Neu_B). High Res. ESMS *m/e* Found: M 613.20810. Calc. for $C_{23}H_{37}N_2O_{17}$: M 613.20923.

11-Azido-1-undecanol (12). A mixture of 11-bromoundecanol **11** (30 g, 119 mmol) and NaN_3 (15.5 g, 238 mmol) in DMF (300 ml) was heated at 100 °C for 2 days, and the temperature of the reaction was raised to 130 °C for 2 h. After cooling to room temperature, EtOAc (300 ml) was added, and insoluble salts were filtered off; the filtrate was concentrated under high vaccum to afford a syrup. The syrup was dissolved in EtOAc (500 ml), and extracted with 10% brine (2 × 100 ml), dried and evaporated to afford the desired azido alcohol (**12**) (24.5 g, 96%). The material was sufficiently pure for use in glycosylations. An analytically pure sample was obtained by chromatography on silica gel (20% AcOEt–hexane). ^{1}H NMR ($CDCl_3$): δ 3.62 (t, 2H, *J* = 6.6, OCH_2), 3.23 (t, 2H, *J* = 7.0, CH_2N_3), 1.52–1.61 (m, 4H, chain), 1.25–1.37 (m, 14H, chain). Found: C, 61.8, H, 10.9, N, 19.3. Calc. for $C_{11}H_{23}N_3O$: C, 61.9, H, 10.9, N, 19.7%.

11-Azidoundecyl β-D-galactopyranoside (15). A mixture of crude perbenzoylated galactopyranosyl bromide (**13**) (prepared from D-galactose by perbenzoylation and subsequent treatment with a solution of 33% HBr–HOAc, the crude bromide **13** contains ~35% of its furanosyl analog) (33.5 g, 52 mmol), azido alcohol (**12**) (14.5 g, 67.8 mmol) and crushed 4 Å molecular sieves (15.0 g) in anhydrous toluene (230 ml) was stirred at −78 °C for 1 h. Silver triflate (20.0 g, 78.0 mmol) was added, and the temperature was allowed to warm up to ~10 °C over one hour. The insoluble material was filtered off and washed with more toluene (3 × 200 ml). The combined filtrate was extracted with a 10% aqueous solution of NH_3 (2 × 500 ml), and concentrated to afford the crude perbenzoate **14**. The mixture was dissolved in anhydrous MeOH (400 ml) containing a catalytic amount of NaOMe, and the mixture was heated to reflux for 1 h. After cooling, the solvent was removed under reduced pressure. Chromatography on silica gel using MeOH–CH_2Cl_2 (0⟶8%) afforded the desired tetrol **15** (12.0 g, 57% yield from D-galactose). $[\alpha]_D$ +16° (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.21 (d, 1H, $J_{1,2}$ = 7.4, H-1), 4.00 (d, 1H, $J_{3,4}$ = 3.1, H-4), 3.81–3.89 (m, 3H, H-6a + H-6b + OCH_a-$H_b(CH_2)_{10}N_3$), 3.63 (dd, 1H, $J_{2,3}$ = 9.6, H-2), 3.56 (dd, 1H, H-3), 3.47–3.52 (m, 2H, H-5 + $OCH_aH_b(CH_2)_{10}N_3$), 3.22 (t, 2H, *J* = 7.0, CH_2N_3), 1.54–1.64 (m, 4H, chain), 1.25–1.39 (m, 14H, chain). Found: C, 54.3, H, 8.9, N, 10.9; M (High Res. ESMS) 398.22632. Calc. for $C_{17}H_{33}N_3O_6$ ($C_{17}H_{33}N_3O_6Na$): C, 54.4, H, 8.9, N, 11.2%; MNa^+, 398.22670.

11-Azidoundecyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (16). A solution of tetraol **15** (11.3 g, 30 mmol), CSA (600 mg) in 2,2-dimethoxypropane (250 ml) was heated to reflux for 3 h. Et_3N (2 ml) was added, and the mixture was concentrated under reduced pressure. After co-evaporation with toluene (2 × 200 ml), the mixture was dissolved in a mixture of MeOH–H_2O (10 : 1, 250 ml) and the reaction was heated to reflux for 3 h. The solution was concentrated and the residue was purified by chromatography on silica gel (40% AcOEt–toluene) to afford the diol **16** (12.0 g, 96%). $[\alpha]_D$ +9.0° (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.16 (d, 1H, $J_{1,2}$ = 8.3, H-1), 4.14 (dd, 1H, $J_{4,5}$ = 2.1, $J_{3,4}$ = 5.6, H-4), 4.08 (dd, 1H, $J_{2,3}$ = 7.5, H-3), 3.98 (m, 1H, H-6a), 3.90 (d‘t’, 1H, $J_{Ha,Hb}$ = 9.5, $J_{Ha,H}$ = 6.7, OCH_a-$H_b(CH_2)_{10}N_3$), 3.80–3.86 (m, 2H, H-5 + H-6b), 3.53 (d‘t’, 1H, $J_{2,OH}$ = 1.6, H-2), 3.48 (d‘t’, $J_{Hb,H}$ = 6.9, $OCH_aH_b(CH_2)_{10}N_3$), 3.23 (t, 2H, *J* = 7.0, CH_2N_3), 2.31 (d, 1H, OH-2), 2.05 (br, 1H, OH-6), 1.54–1.64 (m, 4H, chain), 1.50 (s, 3H, CH_3), 1.24–1.37 (m, 17H, chain + CH_3). Found: C, 57.5, H, 9.0, N, 9.8; M (High Res. ESMS) 438.25746. Calc. for $C_{20}H_{37}N_3O_6$ ($C_{20}H_{37}N_3O_6Na$): C, 57.8, H, 9.0, N, 10.1%; MNa^+ 438.25800.

11-Azidoundecyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (17). To a solution of diol **16** (6.5 g, 15.6 mmol) in anhydrous DMF (120 ml) was added NaH (60% in mineral oil, 3.74 g, 97.5 mmol), the mixture was stirred for 30 min, and cooled to 0 °C. Benzyl bromide (7.4 ml, 62.5 mmol) was added dropwise, and the reaction mixture was left at room temperature for 2 h. MeOH (5 ml) was added to destroy the excess of NaH, and the mixture was concentrated under reduced pressure. The residue was dissolved in AcOEt (350 ml), and the solution was extracted with 10% brine (2 × 100 ml), dried and evaporated. The mixture was purified by silica gel chromatography (3% AcOEt–hexane) to yield **17** as a colorless syrup (8.4 g, 90%). $[\alpha]_D$ +19.8° (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.21–7.40 (m, 10 H, 2 × Bn), 4.84 (d, 1H, *J* = 11.8, Bn), 4.77 (d, 1H, *J* = 11.8, Bn), 4.63 (d, 1H, *J* = 11.9, Bn), 4.54 (d, 1H, *J* = 11.9, Bn), 4.28 (d, 1H, $J_{1,2}$ = 8.1, H-1), 4.10–4.14 (m, 2H, H-3 + H-4), 3.94 (dt, 1H, $J_{Ha,H}$ = 6.4, $J_{Ha,Hb}$ = 9.5, OC*H*aCHb), 3.89 (m, 1H, H-5), 3.78 (dd, 1H, $J_{5,6a}$ = 5.2, $J_{6a,6b}$ = 10.2, H-6a), 3.76 (dd, 1H, $J_{5,6b}$ = 6.9, H-6b), 3.49 (dt, 1H, $J_{Hb,H}$ = 6.9, OCHaC*H*b), 3.36 (m, high order, 1H, H-2), 3.23 (t, 2H, *J* = 7.0, CH_2N_3), 1.64 (m, 2H, OCHaCHbCH_2), 1.57 (m, 2H, C$H_2CH_2N_3$), 1.23–1.41 (m, 14H, chain). Found: C, 68.7, H, 8.6, N, 6.8; (High Res. ESMS) M^+ 618.35136. Calc. for $C_{34}H_{49}N_3O_6$ ($C_{34}H_{49}N_3O_6Na$) C, 68.5, H, 8.3, N, 7.1%; MNa^+ 618.35190.

11-Azidoundecyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (18). A solution of **17** (8.4 g, 14 mmol) in a mixture of 70% HOAc–H_2O (150 ml) was stirred at 85 °C for 4 h, and then concentrated and co-evaporated with toluene (2 × 100 ml). The residue was purified by chromatography on silica gel (30% AcOEt–hexane) to give diol **18** (7.4 g, 95%). $[\alpha]_D$ +5.7° (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.25–7.36 (m, 10H, Bn), 4.93 (d, 1H, J = 11.5, Bn), 4.64 (d, J = 11.5, Bn), 4.07 (s, 2H, Bn), 4.33 (d, $J_{1,2}$ = 7.6, H-1), 3.96 (d, 1H, $J_{3,4}$ = 3.4, H-4), 3.93 (d't', 1H, $J_{Ha,H}$ = 6.4, $J_{Ha,Hb}$ = 9.5, OCH_aH$_b$(CH$_2$)$_{10}$N$_3$), 3.76 (dd, 1H, $J_{5,6a}$ = 5.7, $J_{6a,6b}$ = 10.1, H-6a), 3.71 (dd, 1H, $J_{5,6b}$ = 5.7, H-6b), 3.58 ('t', 1H, H-5), 3.56 (dd, 1H, $J_{2,3}$ = 9.5, H-3), 3.49 (d't', $J_{Ha,H}$ = 6.9, $J_{Ha,Hb}$ = 9.5, OCH_aH$_b$(CH$_2$)$_{10}$N$_3$), 3.46 (dd, 1H, H-2), 3.21 (t, 2H, J = 7.0, CH_2N_3), 1.63 (m, 2H, chain), 1.57 (m, 2H, CH_2), 1.23–1.41 (m, 14H, chain). Found: C, 66.8, H, 8.0, N, 7.4; M (High Res. ESMS) 578.32117. Calc. for $C_{31}H_{45}N_3O_6$ ($C_{31}H_{45}N_3O_6Na$) C, 67.0, H, 8.2, N, 7.6%; MNa^+ 578.32060.

11-Azidoundecyl *O*-(β-D-galactopyranosyl)-(1⟶4)-β-D-glucopyanoside (22). Compound **22** was prepared according to a procedure similar to compound **15** from perbenzoylated lactosyl bromide **20** (10.0 g, 8.82 mmol) (prepared from D-lactose by perbenzoylation and subsequent treatment with a solution of 33% HBr–HOAc), azido alcohol (**12**) (3.76 g, 17.64 mmol) and silver triflate (4.53 g, 17.64 mmol). The pure heptol **22** was obtained by chromatography on silica gel using MeOH–CH_2Cl_2 (0⟶20%) to afford the desired heptol **22** (3.36 g, 73% yield from D-lactose). $[\alpha]_D$ −7.9° (*c* 1.0, CH_3OH). ^{1}H NMR (CD_3OD): δ 4.36 (d, 1H, $J_{1,2}$ = 7.6, H-1_Gal), 4.27 (d, 1H, $J_{1,2}$ = 7.8, H-1_Glc), 3.86–3.91 (m, 2H, H-6a_Glc + OCHaCHb), 3.83 (dd, 1H, $J_{5,6b}$ = 4.1, $J_{6a,6b}$ = 11.9, H-6b_Glc), 3.81 (br d, 1H, H-4_Gal), 3.77 (dd, 1H, $J_{5,6a}$ = 7.5, $J_{6a,6b}$ = 11.5, H-6a_Gal), 3.69 (dd, 1H, $J_{5,6b}$ = 4.6, H-6b_Gal), 3.52–3.59 (m, 4H, H-5_Gal + H-2_Gal + H-4_Glc + OCHaHb), 3.51 (t, 1H, $J_{3,4}$ = 8.9, H-3_Glc), 3.47 (dd, 1H, $J_{2,3}$ = 9.8, $J_{3,4}$ = 3.4, H-3_Gal), 3.39 (ddd, 1H, $J_{5,6a}$ = 2.6, $J_{4,5}$ = 9.3, H-5_Glc), 3.26 (t, 2H, J 7.0, CH_2N_3), 3.23 (dd, 1H, $J_{2,3}$ = 8.7, H-2_Glc), 1.51–1.53 (m, 4H, CH_2_chain), 1.30–1.40 (m, 14H, CH_3 + chain). Found: C, 52.7, H, 8.2, N, 7.9; M (High Res. ESMS) 560.27901. Calc. for $C_{23}H_{43}N_3O_{11}$ ($C_{23}H_{43}N_3O_{11}Na$) C, 53.0, H, 8.3, N, 8.1%; MNa^+ 560.27953.

11-Azidoundecyl *O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1⟶4)-β-D-glucopyanoside (23). Compound **23** was prepared according to a procedure similar to compound **16** from **22** (3.21 g, 6.15 mmol). The pure pentol **23** was obtained by chromatography on silica gel using MeOH–CH_2Cl_2 as eluent (0⟶7.5%) to afford the desired pentol **23** (3.09 g, 87%). $[\alpha]_D$ +9.5° (*c* 0.8, CH_3OH). ^{1}H NMR (CD_3OD): δ 4.35 (d, 1H, $J_{1,2}$ = 8.2, H-1_Gal), 4.27 (d, 1H, $J_{1,2}$ = 7.8, H-1_Glc), 4.18 (dd, 1H, $J_{3,4}$ = 5.5, $J_{4,5}$ = 2.1, H-4_Gal), 4.04 (dd, 1H, $J_{2,3}$ = 7.3, H-3_Gal), 3.93 (ddd, 1H, $J_{5,6b}$ = 4.5, $J_{5,6a}$ = 7.7, H-5_Gal), 3.84–3.89 (m, 2H, H-6a_Glc + OCHaCHb), 3.81 (dd, 1H, $J_{5,6b}$ = 4.1, $J_{6a,6b}$ = 12.1, H-6b_Glc), 3.78 (dd, 1H, $J_{6a,6b}$ = 11.6, H-6a_Gal), 3.74 (dd, 1H, H-6b_Gal), 3.52–3.57 (m, 2H, H-4_Glc +OCHaCHb), 3.51 ('t', 1H, $J_{3,4}$ = 8.9, H-3_Glc), 3.44 ('t', 1H, H-2_Gal), 3.38 (ddd, 1H, $J_{5,6a}$ = 2.5, $J_{4,5}$ = 9.3, H-5_Glc), 3.26 (t, 2H, J 7.0, CH_2N_3), 3.22 (dd, 1H, $J_{2,3}$ = 8.9, H-2_Glc), 1.55–1.64 (m, 4H, CH_2_chain), 1.46 (s, 3H, CH_3), 1.30–1.40 (m, 17H, CH_3 + chain). Found: C, 53.8, H, 8.2, N, 7.0; M (High Res. ESMS) 600.31014. Calc. for $C_{26}H_{47}N_3O_{11}$ ($C_{26}H_{47}N_3O_{11}Na$) C, 54.1, H, 8.2, N, 7.3%; MNa^+ 600.31083.

11-Azidoundecyl *O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-benzyl-β-D-glucopyanoside (24). Compound **24** was prepared according to a procedure similar to compound **17** from **23** (2.50 g, 4.33 mmol), benzyl bromide (5.14 ml, 43.3 mmol) and NaH (60% in mineral oil, 3.32 g, 86.6 mmol). Pure **24** (4.09 g, 92%) was obtained by chromatography on silica gel using AcOEt–hexane as eluent (20⟶35%). $[\alpha]_D$ +15° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.20–7.40 (m, 25H, Bn), 4.90 (d, 1H, J = 10.6, Bn), 4.87 (d, 1H, J = 11.0, Bn), 4.77 (d, 1H, J = 11.9, Bn), 4.72 (d, 1H, J = 10.5, Bn), 4.68 (d, 1H, J = 11.0, Bn), 4.64 (d, 1H, J = 11.8, Bn), 4.55 (d, 1H, J = 12.1, Bn), 4.48 (d, 1H, J = 12.1, Bn), 4.40 (d, 1H, J = 12.2, Bn), 4.39 (d, 1H, $J_{1,2}$ = 8.1, H-1_Gal), 4.35 (d, 1H, $J_{1,2}$ = 7.8, H-1_Glc), 4.30 (d, 1H, J = 12.1, Bn), 4.08 (dd, 1H, $J_{3,4}$ = 5.7, $J_{4,5}$ = 1.7, H-4_Gal), 4.01 ('t', 1H, H-3_Gal), 3.89–3.93 (m, 2H, H-4_Glc + OCHaCHb), 3.78 (dd, 1H, $J_{5,6a}$ = 4.4, $J_{6a,6b}$ = 11.2, H-6a_Glc), 3.72 (dd, 1H, $J_{5,6b}$ = 1.7, H-6b_Glc), 3.63–3.68 (m, 2H, H-5_Gal + H-6a_Gal), 3.54 (t, 1H, $J_{2,3} \approx J_{3,4} \approx$ 9.2, H-3_Glc), 3.46–3.53 (m, 2H, H-6b_Gal + OCHaHb), 3.39 (ddd, 1H, H-5_Glc), 3.36 (dd, 1H, H-2_Glc), 3.33 (dd, 1H, H-2_Gal), 3.21 (t, 2H, J 7.0, CH_2N_3), 1.52–1.65 (m, 4H, CH_2_chain), 1.22–1.40 (m, 20H, 2 × CH_3 + chain). Found: C, 71.5, H, 7.5, N, 3.75; M (ESMS) 1050.6. Calc. for $C_{61}H_{77}N_3O_{11}$ ($C_{61}H_{77}N_3O_{11}Na$) C, 71.25, H, 7.55, N, 4.1%; MNa^+ 1050.6.

11-Azidoundecyl *O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-benzyl-β-D-glucopyanoside (25). Compound **25** was prepared according to a procedure similar to compound **18** from **24** (4.0 g, 3.89 mmol). The pure **25** (3.61 g, 96%) was obtained by chromatography on silica gel using AcOEt–toluene as eluent (20⟶35%). $[\alpha]_D$ +16° (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.19–7.37 (m, 25H, Bn), 4.95 (d, 1H, J = 11.0, Bn), 4.88 (d, 1H, J = 11.0, Bn), 4.79 (d, 1H, J = 11.5, Bn), 4.76 (d, 1H, J = 11.0, Bn), 4.69 (d, 1H, J = 11.0, Bn), 4.65 (d, 1H, J = 11.5, Bn), 4.58 (d, 1H, J = 12.1, Bn), 4.43 (d, 1H, J = 12.3, Bn), 4.42 (d, 1H, J = 11.7, Bn), 4.42 (d, 1H, $J_{1,2}$ = 7.3, H-1_Gal), 4.37 (d, 1H, J = 12.1, Bn), 4.36 (d, 1H, $J_{1,2}$ = 7.7, H-1_Glc), 3.96 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.3, H-4_Glc), 3.92 (d, $J_{3,4}$ = 2.6, H-4_Gal), 3.91 (dt, J = 6.6, J = 9.5, OCHaCHb), 3.79 (dd, 1H, $J_{5,6a}$ = 4.4, $J_{6a,6b}$ = 11.0, H-6a_Glc), 3.74 (dd, 1H, $J_{5,6b}$ = 1.7, H-6b_Glc), 3.59 (dd, 1H, $J_{5,H6a}$ = 6.6, $J_{6a,6b}$ = 9.9, H-6a_Gal), 3.56 (t, 1H, $J_{2,3}$ = 9.0, H-3_Glc), 3.49 (dt, J= 6.8, 9.3, OCHaCHb), 3.48 (dd, 1H, $J_{5,6b}$ = 5.3, H-6b_Gal), 3.37–3.44 (m, 4H, H-2_Glc + H-5_Glc + H-2_Gal + H-3_Gal), 3.33 ('t', 1H, H-5_Gal), 3.23 (t, 2H, J 7.0, CH_2N_3), 1.55–1.67 (m, 4H, CH_2_chain), 1.23–1.42 (m, 14H, chain). Found: C, 70.2, H, 7.1, N, 4.1; M (High Res. ESMS) 1010.51352. Calc. for $C_{58}H_{73}N_3O_{11}$ ($C_{58}H_{73}N_3O_{11}Na$) C, 70.5, H, 7.45, N, 4.25%; MNa^+ 1010.51428.

Methyl *O*-[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1′,9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate]-(2⟶3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (26). To a solution of **8** (0.255 g, 0.271 mmol) and **10** (0.263 g, 0.702 mmol) in dry acetonitrile (3.0 ml), was added powdered 3 Å molecular sieves (0.38 g), and the mixture was stirred for 3 h at room temperature and then cooled to −30 °C. *N*-Iodosuccinimide (0.128 g, 0.569 mmol) and triflic acid (5 µl, 0.057 mmol) were added sequentially and the mixture was stirred for 4 days at −30 °C. Dichloromethane (10 ml) was added, the solids were filtered off. The filtrate was washed with dichloromethane (3 × 10 ml) and the combined organic solution was successively washed with 1.0 M Na_2CO_3 (15 ml) and 20% $Na_2S_2O_3$ (15 ml), dried and concentrated. Column chromatography on silica gel (dichloromethane–methanol 40 : 1) of the residue gave a crude portion of **26** (0.2173 g), which was used without further purification.

Methyl *O*-[5-acetamido-8-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonoate)-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate]-(2⟶3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (27). Compound **26** (0.2173 g) was dissolved in dry methanol (10.0 ml) and 1 M sodium methoxide in methanol was added until pH > 11. The reaction mixture was stirred at room temperature for 2 h and concentrated. The crude residue was dissolved in water (10.0 ml) and

stirred at room temperature for 24 h. The reaction mixture was neutralized with 10% acetic acid and purified by HPLC on a C8 column using water as eluent to give **27** (73.8 mg, 28% from **8**). $[\alpha]_D$ −0.5 (*c* 1.0, methanol). ^{1}H NMR (600 MHz, D_2O) δ 7.35–7.50 (m, 10H, Bn), 4.81 (d, 1H, *J* = 11.1, Bn), 4.77 (d, 1H, *J* = 11.0, Bn), 4.64 (d, 1H, *J* = 11.7, Bn), 4.59 (d, 1H, *J* = 11.5, Bn), 4.39 (d, 1H, $J_{1,2}$ = 7.8, H-1_Gal), 4.17 (dd, 1H, $J_{3,4}$ = 3.1, $J_{2,3}$ = 9.9, H-3_Gal), 4.13 (ddd, 1H, $J_{8,9b}$ = 2.1, $J_{8,9a}$ = 3.3, $J_{7,8}$ = 5.8, H-8_Neu_A), 4.08 (dd, 1H, $J_{9a,9b}$ = 12.0, H-9a_Neu_A), 3.97 (broad d, 1H, H-4_Gal), 3.55–3.90 (m, 15H, H-5_Gal + H-6a_Gal + H-6b_Gal + H-4_Neu_A + H-5_Neu_A + H-7_Neu_A + H-9b_Neu_A + H-4_Neu_B + H-5_Neu_B + H-6_Neu_B + H-7_Neu_B + H-8_Neu_B + H-9a_Neu_B + H-9b_Neu_B), 3.55 (s, 3H, OCH_3), 3.50 (dd, 1H, H-2_Gal), 3.49 (broad d, 1H, $J_{5,6a}$ = 9.9 Hz, H-6a_Neu_A), 2.74 (dd, 1H, $J_{3e,3a}$ = 12.2, H-3e_Neu_A), 2.63 (dd, 1H, $J_{3e,3a}$ = 12.2, H-3e_Neu_B), 2.05, 2.02 (2 × s, 2 × 3H, 2 × NHAc), 1.72 ('t', 1H, H-3a_Neu_B), 1.71 ('t', 1H, H-3a_Neu_A). Found: M (High Res. ESMS) 979.35352. Calc. for $C_{43}H_{60}N_2O_{22}Na$: MNa^+ 979.35354.

Methyl *O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid]-(2⟶8)-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2⟶3)-β-D-galactopyranoside (2). To a solution of compound **27** (73.8 mg, 0.0771 mmol) in dry methanol (10 ml) was added 20% moist palladium hydroxide (81.1 mg) and the mixture was stirred under a hydrogen atmosphere for two days. The catalyst was filtered off and washed with methanol (3 × 10 ml) and then the filtrate was concentrated. The residue was purified on a C18-SepPak cartridge to afford **2** (54.3 mg, 91%). $[\alpha]_D$ +11.7 (*c* 0.6, water). ^{1}H NMR (500 MHz, D_2O) δ 4.46 (d, 1H, $J_{1,2}$ = 8.1, H-1_Gal), 4.25 (dd, 1H, $J_{8,9a}$ = 4.0, $J_{9a,9b}$ = 12.1, H-9a_Neu_A), 4.22 (m, 1H, H-8_Neu_A), 4.15 (dd, 1H, $J_{3,4}$ = 3.2, $J_{2,3}$ = 10.1, H-3_Gal), 4.05 (broad d, 1H, H-4_Gal), 3.65–4.01 (m, 15H, H-5_Gal + H-6a_Gal + H-6b_Gal + H-4_Neu_A + H-5_Neu_A + H-6_Neu_A + H-7_Neu_A + H-9b_Neu_A + H-4_Neu_B + H-5_Neu_B + H-6_Neu_B + H-7_Neu_B + H-8_Neu_B + H-9a_Neu_B + H-9b_Neu_B), 3.65 (s, 3H, OCH_3), 3.60 (dd, 1H, H-2_Gal), 2.85 (dd, 1H, $J_{3e,4}$ = 4.6, $J_{3e,3a}$ = 12.1, H-3e_Neu_A), 2.75 (dd, 1H, $J_{3e,4}$ = 4.5, $J_{3e,3a}$ = 12.2, H-3e_Neu_B), 2.15, 2.10 (2 × s, 2 × 3H, 2 × NHAc), 1.83 ('t', 1H, H-3a_Neu_A), 1.82 ('t', 1H, H-3a_Neu_B). Found: M (High Res. ESMS) 799.25936. Calc. for $C_{29}H_{48}N_2O_{22}Na$: MNa^+ 799.25964.

11-Azido-undecyl *O*-[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylono-1′,9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate]-(2⟶3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (28). To a solution of **8** (0.285 g, 0.303 mmol) and **18** (0.207 g, 0.372 mmol) in dry acetonitrile (2.0 ml) was added powdered 3 Å molecular sieves (0.50 g), and the mixture was stirred for 3 h at room temperature and cooled to −40 °C. *N*-Iodosuccinimide (0.141 g, 0.627 mmol) and triflic acid (5 µl, 0.057 mmol) were added successively and the mixture was stirred for 20 h at −40 °C. The mixture was diluted with dichloromethane (10 ml) and the solids were filtered off and washed with dichloromethane (3 × 10 ml). The combined filtrate was successively washed with 1 M Na_2CO_3 (15 ml), 20% $Na_2S_2O_3$ (15 ml) and sat. brine (10 ml), dried and concentrated. Column chromatography on silica gel (toluene–acetone, 4 : 1 to 3 : 2) of the residue afforded a crude portion of **28** (0.174 g), which was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20–7.35 (m, 10H, Bn), 5.42 (d, 1H, $J_{5,NH}$ = 9.2, NH_Neu_B), 5.37 (ddd, 1H, $J_{3e,4}$ = 5.4, $J_{3a,4}$ = $J_{4,5}$ = 11.0, H-4_Neu_A), 5.33 (dd, 1H, $J_{6,7}$ = 2.1, $J_{7,8}$ = 9.2, H-7_Neu_A), 5.19 (d, 1H, $J_{5,NH}$ = 10.5, NH_Neu_A), 5.11 (ddd, 1H, $J_{8,9a}$ = 2.9, $J_{8,9b}$ = 4.8, H-8_Neu_A), 5.08 (dd, 1H, $J_{6,7}$ = 1.4, $J_{7,8}$ = 7.3, H-7_Neu_B), 5.06 (ddd, 1H, $J_{3e,4}$ = 5.0, $J_{3a,4}$ = $J_{4,5}$ = 10.5, H-4_Neu_B), 4.88 (d, 1H, *J* = 11.2, Bn), 4.59 (d, 1H, *J* = 11.3, Bn), 4.56 (d, 1H, *J* = 11.5, Bn), 4.53 (d, 1H, *J* = 12.0, Bn), 4.40 (dd, 1H, $J_{8,9a}$ = 9.8, $J_{9a,9b}$ = 12.4, H-9a_Neu_B), 4.37 (d, 1H, $J_{1,2}$ = 7.9, H-1_Gal), 4.25 (dd, 2H, $J_{9a,9b}$ = 12.5, $J_{8,9a/b}$ =3.5, H-9a_Neu_A + H-9b_Neu_B), 4.20 (ddd, 1H, H-8_Neu_B), 4.17 (ddd, 1H, $J_{5,6}$ = 10.7, H-5_Neu_A), 3.97–4.06 (m, 3H, H-5_Neu_B + H-6_Neu_B + H-9b_Neu_A), 3.97 (dd, 1H, $J_{3,4}$ = 3.4, $J_{2,3}$ = 10.0, H-3_Gal), 3.94 (dt, 1H, *J* = 6.6, *J* = 6.6, $J_{Ha,Hb}$ = 9.4, OC*H*aHb), 3.87 (dd, 1H, H-6_Neu_A), 3.84 (broad d, 1H, H-4_Gal), 3.80 (s, 3H, COOMe_Neu_B), 3.79 (dd, 1H, $J_{5,6a}$ = 6.0, $J_{6a,6b}$ = 10.3, H-6a_Gal), 3.70 (dd, 1H, $J_{5,6b}$ = 6.1, H-6_Gal), 3.57 (broad dd, 1H, H-5_Gal), 3.52 (dd, 1H, H-2_Gal), 3.51 (dt, 1H, OCHa*H*b), 3.22 (t, 2H, *J* = 7.0, CH_2N_3), 2.45 (dd, 1H, $J_{3a,3e}$ = 14.0, H-3e_Neu_B), 2.40 (dd, 1H, H-3e_Neu_A), 2.07 ('t', 1H, H-3a_Neu_B), 2.09, 2.05, 2.02, 2.01, 1.99, 1.96 (6 × s, 6 × 3H, 6 × OAc), 1.92 ('t', 1H, H-3a_Neu_A), 1.88, 1.87 (2 × s, 2 × 3H, 2 × NHAc), 1.50–1.68 (m, 4H, chain), 1.18–1.40 (m, 14H, chain).

11-Azido-undecyl *O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid]-(2⟶8)-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2⟶3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (29). Compound **28** (0.174 g) was dissolved in dry methanol (6.0 ml) and a solution of 1 M NaOMe in MeOH was added until pH > 11. The reaction mixture was stirred at room temperature for 1 h and concentrated. The crude residue was dissolved in water (6.0 ml) and stirred at room temperature for 24 h. The reaction mixture was neutralized with 10% acetic acid. After concentration, the residue was purified by HPLC on a C8 column (0⟶60%, water–methanol gradient) to give **29** (77.1 mg, 22% from **8**). $[\alpha]_D$ −7.1 (*c* 1.5, methanol). ^{1}H NMR (600 MHz, D_2O) δ 7.35–7.50 (m, 10H, Bn), 4.80 (d, 1H, *J* = 11.2, Bn), 4.77 (d, 1H, *J* = 11.0, Bn), 4.64 (d, 1H, *J* = 11.8, Bn), 4.59 (d, 1H, *J* = 11.5, Bn), 4.46 (d, 1H, $J_{1,2}$ = 7.9, H-1_Gal), 4.15 (dd, 1H, $J_{3,4}$ = 3.2, $J_{2,3}$ = 9.9, H-3_Gal), 4.12 (ddd, 1H, $J_{8,9b}$ = 2.1, $J_{8,9a}$ = 3.3, $J_{7,8}$ = 5.8, H-8_Neu_A), 4.08 (dd, 1H, $J_{9a,9b}$ = 12.5, H-9_Neu_A), 3.97 (broad d, 1H, H-4_Gal), 3.55–3.93 (m, 17H, H-5_Gal + H-6a_Gal + H-6b_Gal + H-4_Neu_A + H-5_Neu_A + H-7_Neu_A + H-9b_Neu_A + H-4_Neu_B + H-5_Neu_B + H-6_Neu_B + H-7_Neu_B + H-8_Neu_B + H-9a_Neu_B + H-9b_Neu_B + OC*H*aHb + OCHa*H*b), 3.50 (broad d, 1H, $J_{5,6a}$ = 10.5 Hz, H-6a_Neu_A), 3.49 (dd, 1H, H-2_Gal), 3.27 (t, 2H, *J* = 7.0, CH_2N_3), 2.74 (dd, 1H, $J_{3e,4}$ = 4.6, $J_{3e,3a}$ = 12.2, H-3e_Neu_A), 2.62 (dd, 1H, $J_{3e,4}$ = 4.5, $J_{3e,3a}$ = 12.1, H-3e_Neu_B), 2.05, 2.02 (2 × s, 2 × 3H, 2 × NHAc), 1.73 ('t', 1H, H-3a_Neu_B), 1.71 ('t', 1H, H-3a_Neu_A), 1.60 (p, 2H, chain), 1.55 (p, 2H, chain), 1.23–1.42 (m, 14H, chain). Found: M (High Res. ESMS) 1160.51140. Calc. for $C_{53}H_{79}N_5O_{22}Na$: MNa^+ 1160.51144.

11-Amino-undecyl *O*-[5-acetamido-8-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonoate)-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate]-(2⟶3)-β-D-galactopyranoside (3). A solution of compound **29** (0.018 g, 15 µmol) and condensed ammonia (50 ml) was cooled to −78 °C. *tert*-butyl alcohol (1.0 ml) and sodium metal (100 mg) were added and the reaction was continued at −78 °C for 4 h; methanol (1.0 ml) was added to quench the reaction. Ammonia was allowed to evaporate, more methanol (15 ml) was added and the mixture was concentrated and co-concentrated with methanol (3 × 15 ml) to a crude residue, which was redissolved in water (15 ml). The pH was adjusted to 8 with 25% acetic acid, and the inorganic salts were removed with a C18 Sep-Pak. Compound **3** (14.6 mg, 99%) was obtained by HPLC employing gradient elution on a C8 column (0⟶60%, water–methanol). $[\alpha]_D$ +3.0 (*c* 1.1, water). ^{1}H NMR (600 MHz, D_2O) δ 4.45 (d, 1H, $J_{1,2}$ = 7.9, H-1_Gal), 4.17 (dd, 1H, $J_{8,9a}$ = 3.8, $J_{9a,9b}$ = 11.9, H-9a_Neu_A), 4.14 (ddd, 1H, $J_{8,9b}$ = 5.6, $J_{7,8}$ = 5.6, H-8_Neu_A), 4.06 (dd, 1H, $J_{3,4}$ = 3.2, $J_{2,3}$ = 9.7, H-3_Gal),

3.96 (broad d, 1H, H-4_Gal), 3.57–3.93 (m, 17H, H-5_Gal + H-6a_Gal + H-6b_Gal + H-4_Neu_A + H-5_Neu_A + H-6_Neu_A + H-7_Neu_A + H-9b_Neu_A + H-4_Neu_B + H-5_Neu_B + H-6_Neu_B + H-7_Neu_B + H-8_Neu_B + H-9a_Neu_B + H-9b_Neu_B + OC*H*aHb + OCHa*H*b), 3.52 (dd, 1H, H-2_Gal), 2.99 (t, 2H, J = 7.5, CH_2NH$_2$), 2.77 (dd, 1H, $J_{3e,4}$ = 4.5, $J_{3e,3a}$ = 12.2, H-3e_Neu_A), 2.68 (dd, 1H, $J_{3e,4}$ = 4.5, $J_{3e,3a}$ = 12.2, H-3e_Neu_B), 2.07, 2.03 (2 × s, 2 × 3H, 2 × NHAc), 1.74 ('t', 1H, H-3a_Neu_B), 1.73 ('t', 1H, H-3a_Neu_A), 1.59–1.63 (m, 4H, chain), 1.28–1.41 (m, 14 H, chain). Found: M (High Res. ESMS) 932.44534. Calc. for $C_{39}H_{70}N_3O_{22}$: MH$^+$ 932.44510.

Methyl *O*-[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylano-1′,9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate]-(2⟶3)-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzyl-β-D-glucopyanoside (30). A mixture containing the acceptor **19** (41 mg, 51 μmol), donor **8** (94 mg, 100 μmol) and 4 Å molecular sieves (300 mg) in anhydrous CH_3CN (1.5 ml) was stirred under Ar overnight and the mixture was cooled to −30 °C. NIS (24 mg, 100 μmol) was added and TfOH (10 μl) was added dropwise 10 min later. After stirring for 3 days at the same temperature, Et_3N (0.5 ml) was added to quench the reaction. The mixture was diluted with CH_2Cl_2 (10 ml) and the insoluble material was filtered off and washed with more CH_2Cl_2 (3 × 10 ml); the combined organic solution was washed with 10% $Na_2S_2O_3$ solution, dried and evaporated. The residue was first purified by chromatography on silica gel (30⟶50% acetone–toluene) to afford compound **30** (31.2 mg), which was impure according to NMR. Pure **30** (20.9 mg, 25%) was obtained by HPLC chromatography on silica gel (0⟶2% MeOH–CH_2Cl_2). $[\alpha]_D$ −9.5° (c 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.19–7.40 (m, 25H, Bn), 5.68 (d, 1H, $J_{5,NH}$ = 9.9, NH_Neu_B), 5.34 (ddd, 1H, $J_{3e,4}$ = 5.3, $J_{3a,4} \approx J_{4,5}$ = 11.2, H-4_Neu_A), 5.33 (dd, 1H, $J_{6,7}$ = 2.4, $J_{7,8}$ = 9.2, H-7_Neu_A), 5.28 (d, 1H, $J_{5,NH}$ = 10.4, NH_Neu_A), 5.14 (ddd, 1H, $J_{8,9a}$ = 2.8, $J_{8,9b}$ = 5.0, H-8_Neu_A), 5.08 (ddd, 1H, $J_{3e,4}$ = 5.1, $J_{3a,4} \approx J_{4,5}$ = 11.0, H-4_Neu_B), 5.04 (dd, 1H, $J_{6,7}$ = 1.8, $J_{7,8}$ = 8.2, H-7_Neu_B), 4.96 (d, 1H, J = 10.4, Bn), 4.84 (d, 1H, J = 11.2, Bn), 4.72 (d, 1H, J = 10.6, Bn), 4.70 (d, 1H, J = 11.5, Bn), 4.69 (d, 1H, J = 11.0, Bn), 4.62 (d, 1H, J = 12.2, Bn), 4.60 (d, 1H, J = 12.6, Bn), 4.46 (d, 1H, $J_{1,2}$ = 7.6, H-1_Gal), 4.46 (d, 1H, J = 12.1, Bn), 4.41 (d, 1H, J = 11.8, Bn), 4.31–4.36 (m, 2H, H-9a_Neu_B + Bn), 4.26 (d, 1H, $J_{1,2}$ = 7.9, H-1_Glc), 4.25 (dd, 1H, $J_{8,9a}$ = 2.7, $J_{9a,9b}$ = 12.8, H-9a_Neu_A), 4.16–4.29 (m, 2H, H-8_Neu_B + H-5_Neu_A), 4.10 (dd, 1H, $J_{8,9b}$ = 3.3, H-9b_Neu_B), 4.06 (ddd, 1H, H-5_Neu_B), 4.02 (dd, 1H, $J_{8,9b}$ = 4.9, $J_{9a,9b}$ = 12.6, H-9b_Neu_A), 4.00 ('t', 1H, $J_{3,4} \approx J_{4,5}$ = 9.5, H-4_Glc), 3.96 (dd, 1H, $J_{5,6}$ = 10.4, H-6_Neu_A), 3.93 (dd, 1H, $J_{5,6}$ = 10.6, H-6_Neu_B), 3.89 (br 'd', 1H, H-4_Gal), 3.83 (dd, 1H, $J_{2,3}$ = 9.3, $J_{3,4}$ = 3.3, H-3_Gal), 3.80 (s, 3H, COOMe_Neu_B), 3.78 (dd, 1H, $J_{5,6a}$ = 4.4, H-6a_Glc), 3.73 (dd, 1H, $J_{5,6b}$ = 1.8, J6a,6b = 11.0, H-6b_Glc), 3.68 (dd, 1H, $J_{5,6a}$ = 7.3, $J_{6a,6b}$ = 9.7, H-6a_Gal), 3.54 (s, 3H, OMe), 3.53 ('t', 1H, $J_{2,3} \approx J_{3,4}$ = 9.3, H-3_Glc), 3.49 (dd, 1H, $J_{2,3}$ = 9.3, H-2_Gal), 3.47 (dd, 1H, $J_{5,6b}$ = 5.3, H-6b_Gal), 3.33–3.38 (m, 3H, H-5_Glc + H-5_Gal + H-2_Glc), 2.40 (dd, 1H, $J_{3a,3e}$ = 13.2, H-3e_Neu_B), 2.39 (dd, 1H, $J_{3a,3e}$ = 13.5, H-3e_Neu_A), 2.10 ('t', 1H, H-3a_Neu_A), 2.09, 2.09, 2.01, 1.99, 1.98, 1.94, (6 × s, 6 × 3H, 6 × OAc),m 1.90 ('t' 1H, H-3a_Neu_B), 1.89, 1.87 (2 × s, 2 × 3H, 2 × OAc). Found: C, 54.3, H, 8.9, N, 10.9; M (High Res. ESMS) 398.22632. Calc. for $C_{17}H_{33}N_3O_6$ ($C_{17}H_{33}N_3O_6Na$): C, 54.4, H, 8.9, N, 11.2%; MNa$^+$ 398.22670.

Methyl *O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid]-(2⟶8)-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2⟶3)-*O*-(β-D-galactopyranosyl)-(1⟶4)-β-D-glucopyanoside (4). To a solution of compound **30** (18 mg, 11 μmol) in anhydrous MeOH (10 ml), was added a solution of NaOMe in MeOH (1.5 M, 0.2 ml), and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in MeOH (5 ml); H_2O (5 drops) was added, and the mixture was stirred for another 24 h. The solution was neutralized with a piece of dry-ice and then concentrated. The residue was dissolved in MeOH (5 ml), 10% Pd(OH)$_2$-C (50 mg) was added and the mixture was stirred under an atmosphere of H_2 for 17 h. The catalyst was filtered off, and washed with more MeOH (3 × 5 ml), and the combined filtrate was concentrated. Compound **4** (9.2 mg, 89%) was obtained by HPLC chromatography on a C18 column using H_2O as eluent. $[\alpha]_D$ +1° (c 0.7, H_2O). ^{1}H NMR (D_2O): δ 4.53 (d, 1H, $J_{1,2}$ = 7.9, H-1_Gal), 4.42 (d, 1H, $J_{1,2}$ = 7.9, H-1_Glc), 4.19 (dd, 1H, $J_{8,9a}$ = 3.9, $J_{9a,9b}$ = 12.3, H-9a_Neu_A), 4.15 (m, 1H, H-8a_Neu_A), 4.10 (dd, 1H, $J_{2,3}$ = 3.1, $J_{3,4}$ = 9.9, H-3_Gal), 4.03 (dd, 1H, $J_{5,6a}$ = 2.2, $J_{6a,6b}$ = 12.3, H-6a_Glc), 3.98 (br d, 1H, H-4_Gal), 3.59–3.93 (m, 19 H, H-3_Glc + H-4_Glc + H-5_Glc +H-6b_Glc + H-5_Gal + H-6a_Gal +H-6b_Gal + H-4_Neu_A + H-5_Neu_A + H-6_Neu_A + H-7_Neu_A + H-9b_Neu_A + H-4_Neu_B + H-5_Neu_B + H-6_Neu_B +H-7_Neu_B +H-8_Neu_B + H-9a_Neu_B + H-9b_Neu_B), 3.59 (s, 3H, OMe), 3.58 (dd, 1H, H-2_Gal), 3.32 ('t', 1H, $J_{2,3} \approx$ 8.9, H-2_Glc), 2.79 (dd, 1H, $J_{3e,4}$ = 4.6, $J_{3e,3a}$ = 12.3, H-3e_Neu_A), 2.69 (dd, 1H, $J_{3e,4}$ = 4.6, $J_{3e,3a}$ = 12.5, H-3e_Neu_B), 2.08, 2.04 (2 × s, 2 × 3H, 2 × Ac), 1.76 ('t', 1H, H-3a_Neu_A), 1.75 ('t', 1H, H-3b_Neu_B). Found: M$^-$ (High Res. ESMS) 468.15414. Calc. for $C_{35}H_{54}N_2O_{27}$: M^{2-} 468.15353.

11-Azidoundecyl *O*-[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylano-1′,9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate]-(2⟶3)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1⟶4)-2,3,6-tri-*O*-benzyl-β-D-glucopyanoside (31). Compound **31** was obtained according to a procedure similar to **30** by reacting acceptor **25** (45 mg, 50 μmol) with donor **8** (94 mg, 100 μmol). Pure **31** (24.0 mg, 29%) was obtained by HPLC chromatography on silica gel (0⟶2% MeOH–CH_2Cl_2). $[\alpha]_D$ −12° (c 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.19–7.41 (m, 25H, Bn), 5.64 (d, 1H, $J_{5,NH}$ = 10.1, NH_Neu_B), 5.34 (ddd, 1H, $J_{3e,4}$ = 5.4, $J_{3a,4} \approx J_{4,5}$ = 10.9, H-4_Neu_A), 5.33 (dd, 1H, $J_{6,7}$ = 1.8, $J_{7,8}$ = 9.0, H-7_Neu_A), 5.24 (d, 1H, $J_{5,NH}$ = 10.2, NH_Neu_A), 5.14 (ddd, 1H, $J_{8,9a}$ = 2.9, $J_{8,9b}$ = 4.9, H-8_Neu_A), 5.08 (ddd, 1H, $J_{3e,4}$ = 5.1, $J_{3a,4} \approx J_{4,5}$ = 10.9, H-4_Neu_B), 5.04 (dd, 1H, $J_{6,7}$ = 1.8, $J_{7,8}$ = 8.1, H-7_Neu_B), 4.95 (d, 1H, J = 10.5, Bn), 4.88 (d, 1H, J = 10.5, Bn), 4.73 (d, 1H, J = 11.0, Bn), 4.71 (d, 1H, J = 11.8, Bn), 4.70 (d, 1H, J = 11.0, Bn), 4.60 (d, 1H, J = 12.2, Bn), 4.59 (d, 1H, J = 11.3, Bn), 4.47 (d, 1H, $J_{1,2}$ = 7.6, H-1_Gal), 4.46 (d, 1H, J = 12.2, Bn), 4.41 (d, 1H, J = 11.8, Bn), 4.34 (d, 1H, $J_{1,2}$ = 7.8, H-1_Glc), 4.29–4.34 (m, 2H, H-9a_Neu_B + Bn), 4.25 (dd, 1H, $J_{9a,9b}$ = 12.7, H-9a_Neu_A), 4.16–4.20 (m, 2H, H-8_Neu_B + H-5_Neu_A), 4.10 (dd, $J_{9a,9b}$ = 12.1, H-9b_Neu_B), 4.06 (ddd, 1H, H-5_Neu_B), 4.02 (dd, 1H, H-9b_Neu_A), 3.98 ('t', 1H, H-4_Glc), 3.96 (dd, 1H, $J_{5,6}$ = 10.7, H-6_Neu_A), 3.88–3.94 (m, 3H, H-6_Neu_B + H-4_Gal + OC*H*aHb), 3.83 (dd, 1H, $J_{2,3}$ = 9.3, $J_{3,4}$ = 3.5, H-3_Gal), 3.80 (s, 3H, COOMe_Neu_B), 3.77 (dd, 1H, $J_{5,6a}$ = 4.7, $J_{6a,6b}$ = 11.5, H-6a_Glc), 3.73 (dd, 1H, $J_{5,6b}$ = 2.3, H-6b_Glc), 3.68 (dd, 1H, $J_{5,6a}$ = 7.2, $J_{6a,6b}$ = 9.6, H-6a_Gal), 3.52 ('t', 1H, $J_{2,3}$ $J_{3,4}$ = 9.0, H-3_Glc), 3.44–3.51 (m, 3H, H-6b_Gal + H-2_Gal + OCHa*H*b), 3.33–3.40 (m, 3H, H-5_Glc + H-5_Gal + H-2_Glc), 3.22 (t, 2H, J 7.0, CH_2N_3), 2.40 (dd, 1H, $J_{3a,3e}$ = 13.5, H-3e_Neu_B), 2.39 (dd, 1H, $J_{3a,3e}$ = 13.5, H-3e_Neu_A), 2.09 ('t' 1H, H-3a_Neu_B), 2.09, 2.06, 2.01, 1.99, 1.98, 1.94 (6 × s, 6 × 3H, 6 × OAc), 1.91 ('t', 1H, H-3a_Neu_A), 1.89, 1.87 (2 × s, 2 × 3H, 2 × OAc), 1.54–1.66 (m, 4H, CH_2_chain), 1.22–1.41 (m, 14H, chain). Found: C, 54.3, H, 8.9, N, 10.9; M (High Res. ESMS) 398.22632. Calc. for $C_{17}H_{33}N_3O_6$ ($C_{17}H_{33}N_3O_6Na$): C, 54.4, H, 8.8, N, 11.2%; MNa$^+$ 398.22670.

11-Aminoundecyl *O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid]-(2⟶8)-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2⟶3)-*O*-(β-D-galactopyranosyl)-(1⟶4)-*O*-β-D-glucopyanoside (5). To a solution of compound **31** (22 mg, 12 μmol) in anhydrous MeOH (10 ml), was added a solution of 1.5 M NaOMe in MeOH (0.2 ml), and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in MeOH (5 ml); H_2O (5 drops) was added, and the mixture was stirred for another 24 h. The solution was neutralized with a piece of dry-ice and then concentrated to dryness. The flask containing the residue was placed in a −78 °C dry-ice bath, and condensed ammonia (~5 ml) was collected with the help of a dry-ice condenser; *t*-BuOH (50 μl) was added to the mixture and a small piece of Na (~100 mg) was added, the solution was maintained to a deep blue color for 4 h at −78 °C. MeOH (0.5 ml) was added to quench the reaction, and the mixture was left at room temperature until all the ammonia evaporated. The residue was dissolved in MeOH (10 ml), and the solution was neutralized with a piece of dry-ice. After evaporation, the residue was pre-purified with a C18 Sep-Pak cartridge to remove most of the inorganic impurities and finally purified by HPLC chromatography on a C18 column (0⟶70% MeOH–H_2O) to afford pure tetrasaccharide **5** (9.7 mg, 73%). $[\alpha]_D$ +2.2° (*c* 1.1, H_2O). ^{1}H NMR (D_2O): δ 4.51 (d, 1H, $J_{1,2}$ = 7.9, H-1_Gal), 4.47 (d, 1H, $J_{1,2}$ = 8.1, H-1_Glc), 4.17 (dd, 1H, $J_{8,9a}$ = 3.8, $J_{9a,9b}$ = 12.3, H-9a_Neu_A), 4.14 (m, 1H, H-8a_Neu_A), 4.07 (dd, 1H, $J_{2,3}$ = 3.1, $J_{3,4}$ = 9.9, H-3_Gal), 3.99 (dd, 1H, $J_{5,6a}$ = 2.4, $J_{6a,6b}$ = 12.3, H-6a_Glc), 3.95 (br d, 1H, H-4_Gal), 3.57–3.93 (m, 21 H, H-3_Glc + H-4_Glc + H-5_Glc +H-6b_Glc + H-5_Gal + H-6a_Gal +H-6b_Gal + H-4_Neu_A + H-5_Neu_A + H-6_Neu_A + H-7_Neu_A + H-9b_Neu_A + H-4_Neu_B + H-5_Neu_B + H-6_Neu_B +H-7_Neu_B +H-8_Neu_B + H-9a_Neu_B + H-9b_Neu_B + OC*H*aHb + OCHa*H*b), 3.55 (dd, 1H, H-2_Gal), 3.29 (dd, 1H, $J_{2,3} \approx$ 9.2, H-2_Glc), 2.98 (t, 2H, *J* = 7.5, CH_2NH_2), 2.77 (dd, 1H, $J_{3e,4}$ = 4.8, $J_{3e,3a}$ = 12.5, H-3e_Neu_A), 2.67 (dd, 1H, $J_{3e,4}$ = 4.6, $J_{3e,3a}$ = 12.5, H-3e_Neu_B), 2.06, 2.02 (2 × s, 2 × 3H, 2 × Ac), 1.74 ('t', 1H, H-3a_Neu_A), 1.73 ('t', 1H, H-3b_Neu_B), 1.58–1.67 (m, 4H, chain), 1.36–1.42 (m, 14H, chain). Found: M (High Res. ESMS) 1095.49762. Calc. for $C_{45}H_{80}N_3O_{27}$: MH^+ 1095.49793.

11-Azidoundecyl *O*-(methyl 5-*N*,*N*-diacetylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2⟶3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (33). A mixture of diol **18** (63.0 mg, 0.113 mmol), sialyl donor **32** (109 mg, 0.189 mmol) and 4 Å molecular sieves (200 mg) in a mixture of anhydrous acetonitrile (1.0 ml) and anhydrous propionitrile (0.5 ml) under argon was cooled to −65 °C. After stirring for 4 h, NIS (54.0 mg, 0.226 mmol) was added, the mixture was stirred for another 30 min. TfOH (10 μl) was added, and the reaction was continued for 1 h. Et_3N (0.5 ml) was added to quench the reaction. The mixture was diluted with AcOEt (50 ml), the insoluble material was filtered off and washed with more AcOEt (2 × 50 ml). The combined organic solution was washed with a 1 : 1 mixture of saturated aqueous $NaHCO_3$ and aqueous 10% $Na_2S_2O_3$ (1 × 50 ml), dried and evaporated. Disaccharide **33** (57 mg, 46%) was obtained by chromatography on silica gel (45% AcOEt–hexane). $[\alpha]_D$ −1.8° (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.47 (ddd, $J_{3e,4}$ = 5.2, $J_{3a,4}$ = 11.2, $J_{4,5}$ = 11.2, H-4_Neu), 5.30 (ddd, 1H, $J_{8,9a}$ = 2.6, $J_{8,9b}$ = 5.5, $J_{7,8}$ = 7.6, H-8_Neu), 5.10 (dd, 1H, $J_{6,7}$ = 1.8, H-7_Neu), 4.86 (dd, 1H, $J_{5,6}$ = 10.2, H-6_Neu), 4.82 (d, 1H, *J* = 11.6, Bn), 4.67 (d, 1H, *J* = 11.6, Bn), 4.58 (d, 1H, *J* = 12.2, Bn), 4.55 (d, 1H, *J* = 12.2, Bn), 4.41 (d, 1H, $J_{1,2}$ = 7.6, H-1_Gal), 4.32 (dd, 1H, $J_{9a,9b}$ = 12.5, H-9a_Neu), 4.16 (t, 1H, H-5_Neu), 4.13 (dd, 1H, $J_{3,4}$ = 3.4, $J_{2,3}$ = 9.3, H-3_Gal), 3.99 (dd, 1H, H-9b_Neu), 3.92 (d't', 1H, $J_{Ha,H}$ = 6.4, $J_{Ha,Hb}$ = 9.3, $OCH_aH_b(CH_2)_{10}N_3$), 3.81 (s, 3H, COOMe), 3.80 ('d', 1H, H-4_Gal), 3.79 (dd, 1H, $J_{5,6a}$ = 5.8, $J_{6a,6b}$ = 9.9, H-6a_Gal), 3.72 (dd, 1H, $J_{5,6b}$ = 5.7, H-6b_Gal), 3.63 ('t', 1H, H-5_Gal), 3.48–3.54 (m, 2H, H-2_Gal+$OCH_aH_b(CH_2)_{10}N_3$), 3.23 (t, 2H, *J* = 7.0, CH_2N_3), 2.71 (dd, 1H, $J_{3e,3a}$ = 13.0, H-3e_Neu), 2.34, 2.26, 2.08, 1.99, 1.92, 1.88 (6 × s, 6 × 3H, 6 × Ac), 1.88 (t, 1H, H-3a_Neu), 1.54–1.66 (m, 4H, chain), 1.20–1.40 (m, 14H, chain). Found: C, 59.2, H, 6.6, N, 5.0; M (High Res. ESMS) 1093.48497. Calc. for $C_{53}H_{74}N_4O_{19}$ ($C_{53}H_{74}N_4O_{19}$Na): C, 59.4, H, 7.0, N, 5.2%: MNa^+ 1093.48450.

Ethyl 2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-1-thio-β-D-galactopyranoside (35). A solution of triol **34** (790 mg, 2.235 mmol), 2,2-dimethoxypropane (1.3 ml) and camphor sulfonic acid (50 mg) in acetone (10 ml) was heated to 80 °C for 4 h. Et_3N (1 ml) was added and the solution was evaporated and co-evaporated with toluene (2 × 50 ml). The residue was dissolved in a mixture of MeOH–H_2O (10 : 1, 22 ml), and the solution was heated to reflux for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by chromatography on silica gel to afford compound **35** (35% AcOEt–hexane, 756 mg, 86%). $[\alpha]_D$ +37.6° (*c* 1.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.83 (br, 2H, Phth), 7.69–7.73 (m, 2H, Phth), 5.19 (d, 1H, $J_{1,2}$ = 10.6, H-1), 4.82 (dd, 1H, $J_{3,4}$ = 5.0, $J_{2,3}$ = 8.9, H-3), 4.33 (dd, 1H, H-2), 4.25 (dd, 1H, $J_{4,5}$ = 1.8, H-4), 4.0–4.06 (m, 2H, H-5 + H-6a), 3.85 (m, high order, 1H, H-6b), 2.69 (dq, 1H, $J_{Ha,Hb}$ = 12.7, $J_{Ha,H}$ = 7.5, SC*H*aHb), 2.59 (dq, 1H, $J_{Hb,H}$ = 7.5, SCHa*H*b), 1.63 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.17 (t, 3H, SCHaHbCH_3). Found: C, 57.9, H, 5.7, N, 3.3; M (High Res. ESMS) 416.11420. Calc. for $C_{19}H_{23}NO_6S$ ($C_{19}H_{23}NO_6SNa$): C, 58.0, H, 5.9, N, 3.6%; MNa^+ 416.11438.

Ethyl 6-*O*-benzoyl-2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-1-thio-β-D-galactopyranoside (36). The alcohol **35** (354 mg, 0.90 mmol) was dissolved in anhydrous pyridine (5 ml) at room temperature, and benzoyl chloride (209 μl, 1.80 mmol) was added. After 1h, MeOH (1 ml) was added, and the mixture was concentrated. The residue was purified by chromatography on silica gel to afford **36** (20% AcOEt–hexane, 434 mg, 97%). $[\alpha]_D$ +53° (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.04 (m, 2H, Bz), 7.81 (br, 2H, Phth), 7.66–7.70 (m, 2H, Phth), 7.54 (m, 1H, Bz), 7.40–7.44 (m, 2H, Bz), 5.21 (d, 1H, $J_{1,2}$ = 10.6, H-1), 4.83 (dd, 1H, $J_{3,4}$ = 5.0, $J_{2,3}$ = 8.9, H-3), 4.66 (dd, 1H, $J_{5,6a}$ = 4.8, $J_{6a,6b}$ = 11.7, H-6a), 4.62 (dd, 1H, $J_{5,6b}$ = 7.1, H-6b), 4.36 (dd, 1H, H-2), 4.32 (dd, 1H, $J_{4,5}$ = 2.2, H-4), 4.31 (ddd, 1H, H-5), 2.65 (dq, 1H, $J_{Ha,H}$ = 7.5, $J_{Ha,Hb}$ = 12.7, SC*H*aHb), 2.59 (dq, 1H, $J_{Hb,H}$ = 7.5, SCHa*H*b), 1.63 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.17 (t, 3H, SCHaHbCH_3). Found: C, 62.6, H, 5.25, N, 3.7; M (High Res. ESMS) 520.14044. Calc. for $C_{26}H_{27}NO_7S$ ($C_{26}H_{27}NO_7SNa$): C, 62.8, H, 5.5, N, 2.8%; MNa^+ 520.14059;

11-Azidoundecyl *O*-(methyl 5-*N*,*N*-diacetylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2⟶3)-[*O*-(6-*O*-benzoyl-2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-β-D-galactopyranosyl)-(1⟶4)]-2,6-di-*O*-benzyl-β-D-galactopyranoside (37). A solution containing the disaccharide acceptor **33** (31 mg, 0.029 mmol) and galactosyl donor **36** (31 mg, 0.062 mmol) in anhydrous acetonitrile (2.0 ml) was stirred with 4 Å molecular sieves (400 mg) for 4 h at room temperature. The mixture was cooled to 0 °C, NIS (17 mg, 0.071 mmol) and triflic acid (10 μl) were added, and the reaction was continued for another 1 h. Et_3N (1.0 ml) was added to quench the reaction. The mixture was diluted with AcOEt (75 ml) and the insoluble material was filtered off and washed with AcOEt (2 × 25 ml). The combined organic solution was extracted with a 1 : 1 mixture of saturated aqueous $NaHCO_3$ and aqueous 10% $Na_2S_2O_3$ (1 × 25 ml), dried over anhydrous Na_2SO_4, and concentrated. The trisaccharide **37** (20.7 mg, 48%) was obtained by chromatography on silica gel (8% acetone–toluene). $[\alpha]_D$ +64° (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.0–8.04 (m, 19H, Ar), 5.42 (ddd, 1H, $J_{3e,4}$ = 5.0, $J_{3a,4}$ = 11.6, $J_{4,5}$ =

10.0, H-4_Neu), 5.23 (dd, 1H, $J_{8,9a}$ =2.6, $J_{8,9b}$ = 5.3, $J_{7,8}$ = 8.0, H-8_Neu), 5.14 (d, 1H, $J_{1,2}$ = 8.6, H-1_GalN), 5.05 (dd, 1H, $J_{3,4}$ = 5.2, $J_{2,3}$ = 8.8, H-3_GalN), 5.0 (dd, 1H, $J_{6,7}$ = 1.8, H-7_Neu), 4.68 (dd, 1H, $J_{5,6}$ = 10.4, H-6_Neu), 4.66 (dd, 1H, $J_{5,6a}$ = 5.6, $J_{6a,6b}$ =11.4, H-6a_GalN), 4.62–4.58 (m, 3H, H-6b_GalN + 2 × Bn), 4.34 (dd, 1H, $J_{4,5}$ = 1.8, H-4_GalN), 4.28 (m, 2H, H-2_GalN + H-5_GalN), 4.26 (d, 1H, $J_{1,2}$ = 7.6, H-1_Gal), 4.15 (dd, 1H, $J_{9a,9b}$ = 12.7, H-9a_Neu), 4.06 (t, 1H, H-5_Neu), 4.05 (dd, 1H, $J_{3,4}$ = 2.7, $J_{2,3}$ = 10.1, H-3_Gal), 3.90 (d, 1H, J = 12.0, Bn), 3.87 (dd, 1H, H-9b_Neu), 3.80 (s, 3H, CO_2Me), 3.73–3.80 (m, 2H, H-6a_Gal + OC*H*aHb), 3.69 (d, 1H, H-4_Gal), 3.66 (dd, 1H, $J_{5,6a}$ = 6.0, $J_{6a,6b}$ = 10.2, H-6b_Gal), 3.56 (d, 1H, J = 12.0, Bn), 3.51 ('t', 1H, $J_{5,6b}$ = 5.8, H-5_Gal), 3.39 (dt, 1H, $J_{Ha,H}$ = 7.0, $J_{Ha,Hb}$ = 9.1, OCHa*H*b), 3.23 (t, 2H, J = 7.0, CH_2N_3), 2.88 (dd, 1H, $J_{3e,3a}$ = 13.0, H-3e_Neu), 2.74 (dd, 1H, H-2_Gal), 2.30, 2.23, 2.04, 1.94, 1.92, 1.65 (6 × s, 6 × 3H, 6 × Ac),1.54–1.66 (m, 5H, H-3a_Neu + chain), 1.18–1.38 (m, 14H, chain). Found: M (ESMS) 1528.5. Calc. for $C_{15}H_{27}O_9NNa$: MNa^+ 1528.6.

11-Aminoundecyl *O*-(5-*N*-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2⟶3)-[*O*-(2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1⟶4)]-β-D-galactopyranoside, disodium salt (6). A solution of trisaccharide **37** (26 mg, 0.013 mmol) in 90% AcOH–H_2O (3 ml) was heated to 70 °C for 1 h. The mixture was concentrated and co-evaporated to dryness with toluene (2 × 20 ml). The residue was dissolved in anhydrous MeOH (10 ml) and a solution of 1 M NaOMe in MeOH (100 µl) was added, the reaction was continued for 4 h at ambient temperature. The solvent was removed under reduced pressure and the residue was dissolved in MeOH (2 ml); H_2O (5 drops) was added, and the solution was stirred at room temperature for 17 h. The mixture was neutralized with Dowex 50W (H^+) resin and concentrated. The residue was dissolved in 1-butanol (3 ml), NH_2NH_2 (1.0 ml) was added, and the mixture was heated to 110 °C for 18 h. After removing the solvent, the residue was dissolved in anhydrous MeOH (5 ml), Ac_2O (0.3 ml) was added, and the reaction was continued for 3 h. The solution was concentrated, the flask containing the residue was placed in a dry-ice bath and liquid ammonia (~10 ml) was collected; a small piece of Na (~50 mg) was added, and the reaction was stirred for 1 h, then quenched with MeOH (1 ml). The ammonia was allowed to evaporate at room temperature and the residue was dissolved in H_2O (5 ml), and then purified by C18 reverse phase chromatography using a gradient of H_2O–MeOH (0⟶60%) as eluent to yield the desired trisaccharide **6** which was lyophilized (5.1 mg, 34% yield overall). $[\alpha]_D$ +9° (*c* 0.3, D_2O). ^1H NMR (CD_3OD): δ 4.85 (d, 1H, $J_{1,2}$ = 8.6, H-1_GalN), 4.28 (d, 1H, $J_{1,2}$ = 7.9, H-1_Gal), 4.15 ('d', 1H, $J_{3,4}$ = 3.1, H-4_Gal), 3.91 (dd, 1H, $J_{2,3}$ = 9.7, H-3_Gal), 3.94 (dd, 1H, $J_{2,3}$ = 10.4, H-2_GalN), 3.51–3.89 (m, 13H, H-8_Neu + H-9a_Neu + H-9b_Neu + H-3_GalN + H-4_GalN + H-5_GalN + H-6a_GalN + H-6b_GalN + H-5_Gal + H-6a_Gal + H-6b_Gal + OC*H*aHb + OCHa*H*b), 3.43 (dd, 1H, $J_{6,7}$ = 2.0, $J_{5,6}$ = 10.3, H-6_Neu), 3.38 (dd, 1H, $J_{7,8}$ = 9.0, H-7_Neu), 3.35 (dd, 1H, H-2_Gal), 2.83 (t, 2H, J = 7.5, CH_2NH_2), 2.73 (dd, 1H, $J_{3e,4}$ = 5.0, $J_{3e,3a}$ = 12.6, H-3e_Neu), 2.01 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.88 (t, 1H, H-3a_Neu), 1.57–1.63 (m, 4H, chain), 1.28–1.42 (m, 14H, chain). Found: M (High Res. ESMS) 866.41087. Calc. for $C_{36}H_{65}N_3O_{17}Na$: MNa^+ 866.41100.

Conjugation of trisaccharides 3 and 6 to sepharose matrix. *Conjugation with trisaccharide 3.* NHS-activated sepharose gel (6.6 ml dry volume) was placed in a filtration flask, and was successively washed with cold 1.0 mM HCl (100 ml) and a solution of PBS buffer (pH 7.0, 0.05 M, 20 ml). The wet gel was transferred into a flask, a solution of compound **3** (6.6 mg) in the same buffer (3.3 ml) was added and the suspension was shaken for 18 h at room temperature; the resin was filtered off and washed with the same buffer as above (2 × 10 ml). The combined filtrate was lyophilized, and purified by HPLC as previously described to afford compound **3** (2.4 mg), which gives a coupling efficiency of ~65%.

The gel linked with trisaccharide **3** was suspended in a PBS buffer (pH 7.0, 0.05 M, 3.3 ml), ethanolamine (0.33 ml) was added and the mixure was gently shaken for 3 h. The gel was filtered off and successively washed with a cold potassium biphthalate buffer (pH 4, 0.05 M, 3 × 7 ml) and a PBS buffer (pH 8, 0.05 M, 3 × 7 ml); this washing cycle was repeated three times and the gel was collected and stored in 20% aqueous ethanol. The calculated trisaccharide incorporation density is 0.65 µmol **3**/ml gel.

Conjugation with trisaccharide 6. Trisaccharide **6** was conjugated to the NHS-activated sepharose gel as above, starting from **6** (5.0 mg) and gel (6.6 ml dry volume). Unreacted compound **6** (2.3 mg) was recovered, resulting a coupling efficiency of ~54%; the calculated trisaccharide incorporation density is 0.64 µmol **6**/ml gel

Conjugation of trisaccharides 3, 5 and 6 to BSA. *Conjugation with trisaccharide 3.* Amine **3** (6.13 mg, 6.28 µmol) was dissolved in a 1 : 1 solution of ethanol–water (2 ml), and 3,4-diethoxy-3-cyclobutene-1,2-dione (12 µl, 81.9 µmol) was added. A 1 M solution of Na_2CO_3 was added in 5 µl aliquots, until TLC analysis indicated the free amine had been completely converted into a faster moving product. The reaction mixture was then concentrated and purified by HPLC (C8 column, water–methanol gradient) to give the intermediate squarate adduct (5.63 mg, 82%). ^1H NMR (500 MHz, D_2O) δ 4.75 (m, HDO and CH_3CH_2O_Squarate), 4.45 (d, 1H, $J_{1,2}$ = 7.9, H-1_Gal), 4.17 (dd, 1H, $J_{8,9a}$ = 3.8, $J_{9a,9b}$ = 11.6, H-9a_Neu_A), 4.15 (ddd, 1H, $J_{8,9b}$ = 6.0, $J_{7,8}$ = 6.0, H-8_Neu_A), 4.07 (dd, 1H, $J_{3,4}$ = 3.2, $J_{2,3}$ = 9.7, H-3_Gal), 3.96 (broad d, 1H, H-4_Gal), 3.57–3.94 (m, 17H, H-5_Gal + H-6a_Gal + H-6b_Gal + H-4_Neu_A + H-5_Neu_A + H-6_Neu_A + H-7_Neu_A + H-9b_Neu_A + H-4_Neu_B + H-5_Neu_B + H-6_Neu_B + H-7_Neu_B + H-8_Neu_B + H-9a_Neu_B + H-9b_Neu_B + OC*H*aHb + OCHa*H*b), 3.53 (dd, 1H, H-2_Gal), 3.50 (t, 2H, J = 6.9, CH_2NHR), 2.78 (dd, 1H, $J_{3e,4}$ = 4.7, $J_{3e,3a}$ = 12.2, H-3e_Neu_A), 2.68 (dd, 1H, $J_{3e,4}$ = 4.2, $J_{3e,3a}$ = 12.2, H-3e_Neu_B), 2.07, 2.04 (2 × s, 2 × 3H, 2 × NHAc), 1.75 ('t', 1H, H-3a_Neu_B), 1.74 ('t', 1H, H-3a_Neu_A), 1.58–1.66 (m, 4H, chain), 1.39–1.48 (m, 3H, CH_3CH_2O_Squarate), 1.25–1.39 (m, 14 H, chain). Found: M (High Res. ESM) 1078.44355. Calc. for $C_{45}H_{73}N_3O_{25}Na$: MNa^+ 1078.44309. The adduct (5.63 mg, 5.3 µmol) was dissolved in a potassium bicarbonate–sodium borate buffer (pH 9, 1.5 ml) and BSA (34.5 mg, 0.52 µmol) was added. The mixture was stirred at room temperature for 3 days, diluted with water and dialyzed against water (1 l). 10% Sodium azide (1 ml) was added to prevent bacterial growth. The dialysis water was changed three times and the solution was freeze-dried to give the BSA glycoconjugate (36.5 mg). MALDI-TOF-MS showed an average mass of 72535 dalton, which corresponds to an average of 6.0 trisaccharides per BSA.

Conjugation with tetrasaccharide 5. Diethyl squarate (0.9 µl, 6.1 µmol) was added to a solution of tetrasaccharide **5** (7.0 mg, 6.4 µmol) in anhydrous MeOH (1.0 ml). After stirring overnight, the reaction mixture was concentrated. The residue was dissolved in a $Na_2B_4O_7$–$KHCO_3$ buffer (pH 9, 5.0 ml), BSA (35.0 mg, 0.53 µmol) was added and the mixture was stirred for 3 days at room temperature. The mixture was diluted with H_2O (15 ml), and the solution was dialyzed against pure H_2O (3 × 1 l), lyophilized to give the desired BSA conjugate (41 mg). MALDI-TOF-MS indicated the conjugate had an average mass of 76259 daltons, which corresponds to an average of 8.4 trisaccharides per BSA.

Conjugation with trisaccharide 6. Trisaccharide **6** was conjugated to the same gel according to the same procedure as for **5**, starting from **6** (6.0 mg, 7.1 µmol) and diethyl squarate (0.9 µl, 6.1 µmol). The intermediate squarate adduct was reacted with

BSA (23.5 mg, 0.36 μmol) in the same buffer as **5** to afford the desired BSA conjugate (28.0 mg). MALDI-TOF-MS indicated the conjugate had an average mass of 79534 daltons, which corresponds to an average of 14.2 trisaccharides per BSA.

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References

1 S. Salloway, L. A. Mermel, M. Seamans, G. O. Aspinall, J. E. Nam Shin, L. A. Kurjanczyk and J. L. Penner, *Infect. Immun.*, 1996, **64**, 2945–2949.
2 I. Wirguin, L. Suturkova-Milosevic, P. Della-Latta, T. Fisher, R. H. Brown and N. Latov, *Ann. Neurol.*, 1994, **35**, 698–703.
3 N. Yuki, T. Taki, M. Takahashi, K. Saito, H. Yoshino, T. Tai, S. Handa and T. Miyatake, *Ann. Neurol.*, 1994, **36**, 791–793.
4 B. C. Jacobs, H. Endtz, F. G. van der Meche, M. P. Hazenberg, H. A. Achtereekte and P. A. van Doorn, *Ann. Neurol.*, 1995, **37**, 260–264.
5 N. Yuki, *J. Infect. Dis.*, 1997, **176**(Suppl 2), S150–153.
6 M. M. Prendergast, H. J. Willison and A. P. Moran, *Infect. Immun.*, 1999, **67**, 3698–3701.
7 H. J. Willison and G. M. O'Hanlon, *J. Neuroimmunol.*, 1999, **100**, 3–12.
8 N. Yuki, H. Yoshino, S. Sato, K. Shinozawa and T. Miyatake, *Muscle Nerve*, 1992, **15**, 899–903.
9 A. Chiba, S. Kusunoki, T. Shimuzu and I. Kanazawa, *Ann. Neurol.*, 1992, **31**, 677–679.
10 H. J. Willison, A. AlMemar, J. Veitch and D. Thrush, *Neurology*, 1994, **44**, 2395–2398.
11 M. Roberts, H. J. Willison, A. Vincent and J. Newsom-Davis, *Lancet*, 1994, **343**, 454–455.
12 H. J. Willison, G. M. O'Hanlon, G. Paterson, J. Veitch, G. Wilson, M. Roberts, T. Tang and A. Vincent, *J. Clin. Invest.*, 1996, **97**, 1155–1164.
13 B. C. Jacobs, G. M. O'Hanlon, E. G. Breedland, J. Veitch, P. A. van Doorn and H. J. Willison, *J. Neuroimmunol.*, 1997, **80**, 23–30.
14 G. M. O'Hanlon, G. J. Paterson, J. Veitch, G. Wilson and H. J. Willison, *Acta Neuropathol.*, 1998, **95**, 605–616.
15 T. W. Ho, H. J. Willison, I. Nachamkin, C. Y. Li, J. Veitch, H. Ung, G. R. Wang, R. C. Liu, D. R. Cornblath, A. K. Asbury, J. W. Griffin and G. M. McKhann, *Ann. Neurol.*, 1999, **45**, 168–173.
16 N. Yuki, K. Susuki and K. Hirata, *Neurology*, 2000, **54**, 1851–1853.
17 A. F. Hahn, *Lancet*, 1998, **352**, 635–641.
18 M. Fisher, *N. Engl. J. Med.*, 1956, **225**, 57–65.
19 J. P. ter Bruggen, F. G. A. van der Meche, A. E. J. deJager and C. H. Polman, *Muscle Nerve*, 1998, **21**, 239–242.
20 J. H. Rees, S. E. Soudain, N. A. Gregson and R. A. C. Hughes, *N. Engl. J. Med.*, 1995, **333**, 1374–1379.
21 B. Buchwald, A. Weishaupt, K. Toyka and J. Dudel, *Eur. J. Neurosci.*, 1998, **10**, 281–290.
22 J. J. Plomp, P. C. Molenaar, G. M. O'Hanlon, B. C. Jacobs, J. Veitch, M. R. Daha, P. A. van Doorn, F. G. van der Meche, A. Vincent, B. P. Morgan and H. J. Willison, *Ann. Neurol.*, 1999, **45**, 189–199.
23 C. S. Goodyear, G. M. O'Hanlon, J. J. Plomp, E. R. Wagner, I. Morrison, J. Veitch, L. Cochrane, R. W. Bullens, P. C. Molenaar, J. Conner and H. J. Willison, *J. Clin. Invest.*, 1999, **104**, 697–708.
24 S. Kusunoki, S. Hitoshi, K.-I. Kaida, M. Arita and I. Kanazawa, *Ann. Neurol.*, 1999, **45**, 400–403.
25 N. Yuki, T. Taki, F. Inagaki, T. Kasama, M. Takahashi, K. Saito, S. Handa and T. Miyatake, *J. Exp. Med.*, 1993, **178**, 1771–1775.
26 G. Ritter, S. R. Fortunato, L. Cohen, Y. Noguchi, E. M. Bernard, E. Stockert and L. J. Old, *Int. J. Cancer*, 1996, **66**, 184–190.
27 G. O. Aspinall, A. G. McDonald, T. S. Raju, H. Pang, A. P. Moran and J. L. Penner, *Eur. J. Biochem.*, 1993, **213**, 1017–1027.
28 G. O. Aspinall, A. G. McDonald, H. Pang, L. A. Kurjanczyk and J. L. Penner, *Biochemistry*, 1994, **33**, 241–249.
29 G. O. Aspinall, C. M. Lynch, H. Pang, R. T. Shaver and A. P. Moran, *Eur. J. Biochem.*, 1995, **213**, 570–578.
30 J. L. Penner and G. O. Aspinall, *J. Infect. Dis.*, 1997, **176**(Suppl 2), S135–138.
31 A. Chiba, S. Kusunoki, H. Obata, R. Machinami and I. Kanazawa, *Brain Res.*, 1997, **745**, 32–36.
32 H. J. Willison and G. M. O'Hanlon, *J. Neuroimmunol.*, 1993, **46**, 105–112.
33 H. J. Willison, G. M. O'Hanlon, G. Paterson, C. P. O'Leary, J. Veitch, G. Wilson, M. Roberts, T. Tang and A. Vincent, *J. Infect. Dis.*, 1997, **176**(Suppl 2), S144–149.
34 G. M. O'Hanlon, J. J. Plomp, M. Chakrabarti, I. Morrison, E. R. Wagner, C. S. Goodyear, X. Yin, B. D. Trapp, J. Conner, P. C. Molenaar, S. Stewart, E. G. Rowan and H. J. Willison, *Brain*, 2001, **124**, 893–906.
35 G. M. O'Hanlon, R. W. Bullens, J. J. Plomp and H. J. Willison, *Neurochem. Res.*, 2002, **27**, 697–709.
36 R. Roy and R. A. Pon, *Glycoconjugate J.*, 1990, **7**, 3–12.
37 H. Ishida, Y. Ohta, Y. Tsukada, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, 1993, **246**, 75–88.
38 J. Dahmen, G. Gnosspelius, A. Larsson, T. Lave and G. Noori, *Carbohydr. Res.*, 1985, **138**, 17–28.
39 H. Paulsen, T. Hasenkamp and M. Paal, *Carbohydr. Res.*, 1985, **144**, 45–56.
40 J. R. Mota, D. Mostowicz, C. Ortiz, M. A. Pradera and I. Robina, *Carbohydr. Res.*, 1994, **257**, 305–316.
41 F. W. Lichtenthaler, E. Kaji and S. Weprek, *J. Org. Chem.*, 1985, **50**, 3505–3515.
42 A. V. Demchenko and G. J. Boons, *Chem. Eur. J.*, 1999, **5**, 1278–1283.
43 J. Zhang, A. Otter and D. R. Bundle, *Bioorg. Med. Chem.*, 1996, **4**, 1989–2002.
44 V. P. Kamath, P. Diedrich and O. Hindsgaul, *Glycoconjugate J.*, 1996, **13**, 315–319.
45 H. J. Willison, K. Townson, J. Veitch, J. Boffey, N. Isaacs, S. Andersen, P. Zhang, C.-C. Ling and D. R. Bundle, *Brain*, 2004, **127**, 1–12.
46 A. N. Houghton, D. Mintzer, C. Cordon-Cardo, S. Welt, B. Fliegel, S. Vadhan, E. Carswell, M. R. Melamed, H. F. Oettgen and L. J. Old, *Proc. Natl. Acad. Sci. U. S. A.*, 1985, **82**, 1242–1246.