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GM3, GM2 and GM1 mimics designed for biosensing: chemoenzymatic synthesis, target affinities and 900 MHz NMR analysis

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Abstract—Undec-10-enyl, undec-10-ynyl and 11-azidoundecyl glycoside analogues corresponding to the oligosaccharides of human gangliosides GM3, GM2 and GM1 were synthesized in high yields using glycosyltransferases from *Campylobacter jejuni*. Due to poor water solubility of the substrates, the reactions were carried out in methanol–water media, which for the first time were shown to be compatible with the *C. jejuni* α-(2→3)-sialyltransferase (CST-06) and β-(1→4)-*N*-acetylgalactosaminyltransferase (CJL-30). Bioequivalence of our synthetic analogues and natural gangliosides was examined by binding to *Vibrio cholerae* toxin and to the B subunit of *Escherichia coli* heat-labile enterotoxin. This bioequivalence was confirmed by binding mouse and human monoclonal antibodies to GM1 and acute phase sera containing IgM and IgG antibodies to GM1 from patients with the immune-mediated polyneuropathy Guillain–Barré syndrome. The synthesized compounds were analyzed by 1D and 2D 900 MHz NMR spectroscopy. TOCSY and DQF-COSY experiments in combination with ^{13}C–^{1}H correlation measurements (HSQC, HMBC) were carried out for primary structural characterization, and a complete assignment of all ^{1}H and ^{13}C chemical shifts is presented.

Keywords: Enzymatic synthesis; GM1 mimics; Guillain–Barre syndrome; NMR

1. Introduction

The development of high-affinity mimics of carbohydrates associated with important recognition events has attracted a great deal of attention as a way to develop therapeutic agents[1–7] with good stability and synthetic availability.[1–12] Particular interest is focused on gangliosides,[13,14] which are a.o. important targets for auto-antibodies causing immune-mediated forms of polyneuropathy and for bacterial toxins that cause gastroenteritis including cholera and travellers diarrhoea.[15] For example, *Vibrio cholerae* toxin (CT) binds with high affinity to GM1-ganglioside, whereas *Escherichia coli* heat-labile enterotoxin (LT), apart from binding to

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GM1, also binds (though to a lesser extent) to GM2 and other glycolipid receptors.[16,17]

Guillain–Barré syndrome (GBS),[18] the most frequent cause of acute neuromuscular paralysis that can be triggered by *Campylobacter jejuni* infection, was shown to be a true case of molecular mimicry:[19,20] it has its origin in molecular similarities between the carbohydrate part of *C. jejuni* cell wall lipooligosaccharides and the oligosaccharide sequence of the gangliosides in the nerve tissue.[21,22] Apart from causing diseases, such ganglioside—antibody recognition events can also be the basis for cure and diagnostics.[23] However, the success of natural carbohydrates as therapeutic agents and diagnostic tools is presently limited. Thus, serum auto-antibodies to gangliosides in patients with GBS and other forms of immune-mediated polyneuropathy are usually detected by ELISA-based tests. The detection of these antibodies is, however, restricted by the use of bovine brain-derived gangliosides in these assays, which are difficult to purify, expensive, potentially infected and usually contaminated with other glycolipids. These drawbacks may partly explain the variation and limited sensitivity and specificity of the current ELISA to detect the antibodies.[24]

Consequently, pure and structurally analogous synthetic mimics of gangliosides would have significant advantages over their parent structures. The formation of such mimics, and in general of compounds that are able to block glycan recognition does, however, still remain a major challenge.[25] Recently, a range of synthetic GM1 mimics, in which the oligosaccharide part was modified to make them more straightforward to be chemically synthesized,[26,27] were examined in binding to anti-GM1 GBS-related antibodies.[28] However, in both solution inhibition and immunoadsorption studies the naturally derived GM1 pentasaccharide (GM1 lacking the ceramide tail) was found to be greatly superior to the investigated mimics.[28] As a result, we direct our efforts towards the synthesis of mimics with the *authentic GM1 oligosaccharide moiety* as the ligand for binding to cholera toxin[29] and other targets.

The specific interactions between such GM1, GM2 and GM3 ganglioside analogues and GBS-related antibodies open the way for biosensing as a diagnostic tool for GBS, for example, via semiconductor surfaces[30] that have been covalently modified with specific oligosaccharides.[31,32] For the preparation of such monolayers, we developed an extremely mild photochemical method (visible light, room temperature),[33,34] which allows for the attachment of labile bio(macro)molecules such as saccharides, DNA and proteins, if their synthesis with appropriate linkers would be feasible. Linkers of choice for such attachments are ω-alkenyl or ω-alkynyl tails with the length of at least 10 carbon atoms.[33–39] In addition, the ω-alkynyl tail is an excellent tool for attachment via cycloaddition 'click' reactions.[40–42]

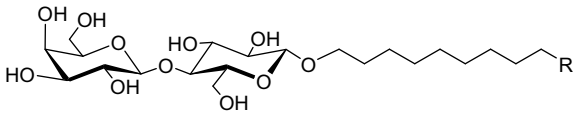
However, reported methods for the efficient preparation of sialylated ganglioside structures are scarce,[43] primarily due to synthetic problems imposed by the properties of the glycosidic linkage involving sialic acid.[44] Recently, the synthesis of a variety of biologically relevant sialyl oligosaccharides was facilitated by a number of chemoenzymatic strategies using bacterial glycosyltransferases.[45–51] Thus, several glycosyltransferase genes from *C. jejuni* have been expressed in *E. coli* and methods for the efficient synthesis of 2-azidoethyl glycosides corresponding to the oligosaccharides of GD3, GT3, GM2, GD2, GT2, GM1 and GD1a were developed with a water-soluble 2-azidoethyl lactoside as starting compound.[52,53] For the application of such materials into sensing devices, attachment of other than highly water-soluble chains would, however, be a big step forward. Given the poor water solubility and gel-forming tendency of lactosides that have a long hydrophobic chain attached to their anomeric oxygen, this requires a better solubilizing yet still glycosyltransferase-compatible medium. In addition, since the literature contains mutually varying, incompatible sets of assignments of the various NMR peaks of the natural[54–57] and synthetic[27,44,52,58] ganglioside structures, a detailed and unambiguous assignment of the structure of GM1 and its precursors is desirable.

The present paper shows that in appropriate methanol/water mixtures amphiphilic lactosides are excellent substrates for the above mentioned set of glycosyltransferases which, in their turn, for the first time were shown to be active in methanol–water media. As a result we subsequently report here the chemoenzymatic synthesis of ω-undecenyl, ω-undecynyl and 11-azidoundecyl glycosides of GM3, GM2 and GM1. In addition, we show the bioequivalence of natural GM1 ganglioside and its undec-10-enyl mimic via a series of binding/receptor studies, which points to the potential of these mimics in sensor and related applications. Finally, we report for the first time a complete and ab initio assignment of all ^{1}H and ^{13}C chemical shifts in the GM3, GM2 and GM1 oligosaccharide moieties via a series of one- and two-dimensional 900 MHz NMR spectra of ω-undecenyl gangliosides.

2. Results and discussion

2.1. Synthesis of GM3, GM2 and GM1 mimics with long (C11) hydrophobic tails

The chemoenzymatic synthesis of glycosides with glycosyltransferases from *C. jejuni*[52] was explored in terms of substrate specificity (presence of a long hydrophobic chain rather than a hydrogen atom[53] or a short alkyl chain[52,59] at the aglycone end) and solvent tolerance. Thus, undec-10-enyl β-lactoside **1**, undec-10-ynyl β-lact-



1 : R= $CH{=}CH_2$
2 : R= $C{\equiv}CH$
3 : R= $CH_2CH_2\text{-}N_3$

Figure 1. Compounds used for enzymatic modifications.

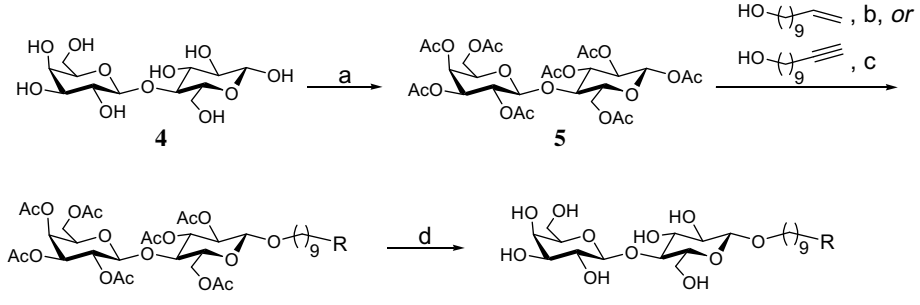
oside **2** and 11-azidoundecyl β-lactoside **3** (Fig. 1) were used for the enzymatic modifications in this study.

Lactoside **3** was synthesized as described previously.[44] Synthesis of **1** and **2** was accomplished in three steps starting from D-lactose **4** (Scheme 1). Lactose octaacetate **5**, prepared by the acetylation of D-lactose, was glycosidated with 10-undecen-1-ol or 10-undecyn-1-ol to give heptaacetyl undecenyl lactoside **6**[60] and heptaacetyl undecynyl lactoside **7**, respectively. The peracetylated lactosides **6** and **7** after isolation were deprotected according to the standard Zemplén procedure[61] to afford heptaols **1** and **2**.[62] Unlike reported ~1:9 mixture of undecenyl α- and β-lactosides,[63] compound **1** was a 100% β-isomer, as characterized by NMR (vide infra). Lactosides **1** and **2** did not require a purification step and were directly used for further transformations.

Compounds **1**–**3** could be converted in a series of novel reaction steps and by a modified procedure to the respective GM3, GM2 and GM1 mimics; the overall reaction scheme is depicted in Scheme 2. Contrary to the previously reported 2-azidoethyl lactoside[52] and other synthetic substrates for glycosyltransferases,[52,59] compounds **1**, **2** and **3** are poorly soluble in water: they form gels, and as such are not amenable for enzymatic modification. The solubility properties of compound **1** appeared to be different from those described before.[63] Thus, it dissolved readily in methanol while clearly forming gels in water. To obtain a suitable medium for the reactions with sialyltransferase, compounds **1**, **2** and **3** were first dissolved in 100% MeOH. After the addition of aqueous solutions of the other components, we obtained a mixture of methanol/water (25/75 v/v) that was compatible with CST-06, the sialyltransferase used to add NeuAc. The mixture of respective compound **1**, **2** or **3**, sialyltransferase CST-06 and CMP-NeuAc was kept at 37 °C for 1 h, after which TLC analysis unambiguously showed that the lactoside was converted completely. In a separate experiment, the target GM3-mimic **8** was subsequently bound on a Sep-Pak column, which was then washed with H_2O to elute hydrophilic compounds (such as the buffer and the nucleotide) and finally the GM3-analogue **8** was eluted with MeOH in 92% yield (Scheme 2).

For the synthesis of GM2-mimics **11**, **12** and **13**, we added the different components directly to the corresponding GM3 reaction mixture which resulted in a final concentration of 10% (v/v) of methanol. Compound **8**, **9** or **10** was in this medium reacted with the in situ-generated UDP-GalNAc in a one-pot mixture containing UDP-GlcNAc, the UDP-GlcNAc 4′-epimerase (CPG-13) and the β-(1,4)-*N*-acetylgalactosaminyltransferase (CJL-30). The corresponding product, GM2-mimic **11**, **12** or **13**, was bound to a Sep-Pak column as described above for GM3-mimics and eluted with MeOH. For compounds **11**, **12** and **13**, quantitative yields (from starting lactosides) were obtained.

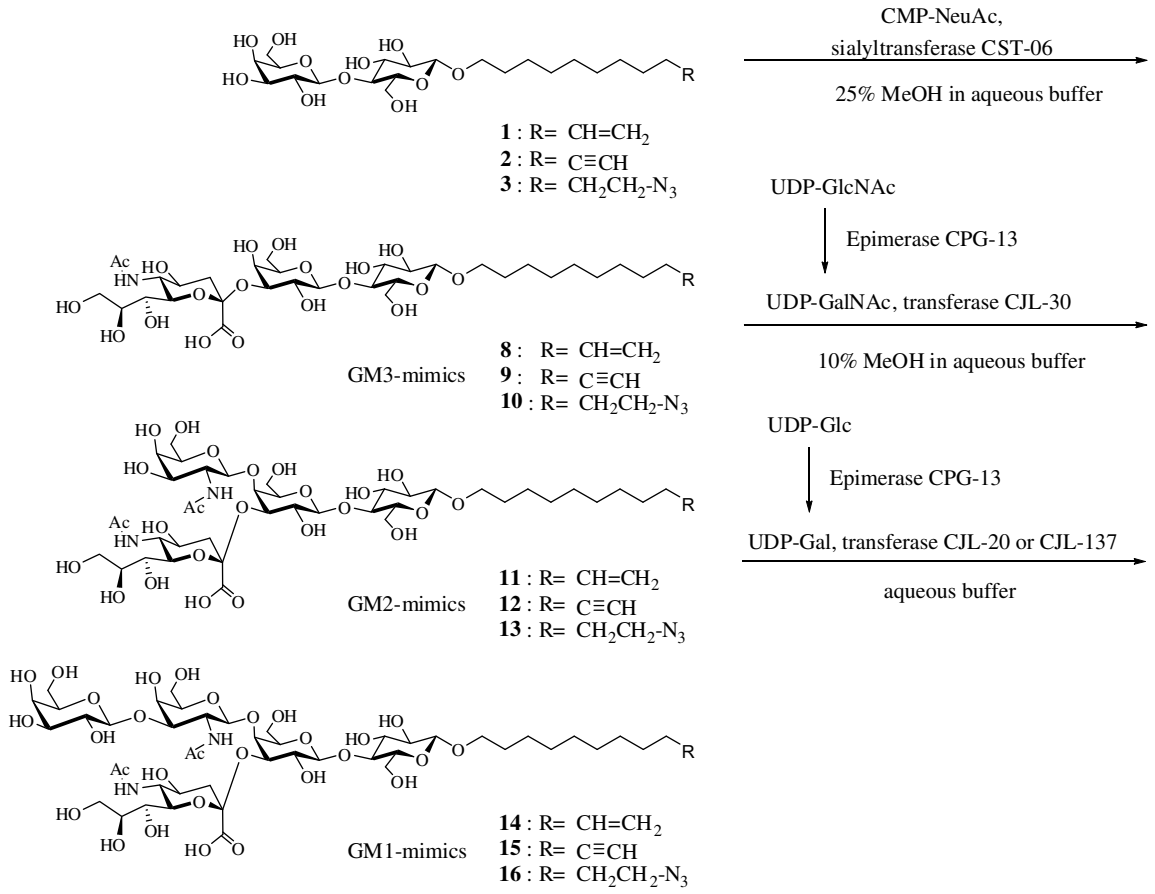
The GM2 analogues **11**, **12** and **13** were further elongated in an aqueous solution to the GM1 mimics **14**, **15** and **16**, respectively. To that end, compound **11** reacted with UDP-Gal and β-(1,3)-galactosyltransferase CJL-20, while compounds **12** and **13** were converted in a one-pot mixture containing UDP-Glc, epimerase CPG-13 and β-(1,3)-galactosyltransferase CJL-137. Corresponding GM1 mimics were recovered after purification in high yields (94% for compound **14**, 99% for compound **15** and 75% for compound **16**). The identity of these GM3, GM2 and GM1 mimics was proved by a series of 900 MHz NMR experiments (vide infra, part **2.3**).



6 : R= $CH{=}CH_2$
7 : R= $C{\equiv}CH$

1 : R= $CH{=}CH_2$
2 : R= $C{\equiv}CH$

Scheme 1. Synthesis of heptaols **1** and **2**. Reagents and conditions: (a) Ac_2O, NaOAc, reflux, 4 h; (b) $SnCl_4$ in dry CH_2Cl_2, rt, overnight, 56%; (c) $BF_3{*}Et_2O$ in dry CH_2Cl_2, 0 °C to rt, overnight, 40%; (d) $NaOCH_3$ in MeOH, rt (overnight), or rt to reflux (15 min), 100%.



Scheme 2. Enzymatic syntheses of GM3, GM2 and GM1 mimics.

2.2. Evaluation of the receptor-binding affinity of GM1 analogues

The synthesized GM1 mimic **14** has been tested for its bioequivalence to the natural bovine brain GM1. In order to investigate this, **14** was firstly subjected to ELISA titration studies with the B subunit of *E. coli* heat-labile enterotoxin (LT-B). It is well known that binding of CT and LT to gangliosides is mediated through their respective pentameric B subunits.[64] Because of the high binding affinities for gangliosides, these B subunits are ideal tools to study structural similarities between natural and synthetic analogues.

The effectiveness of **14** in binding LT-B is demonstrated in Figure 2, which also indicates that the undec-10-enyl GM1 analogue **14** appeared to be in general even better than the bovine GM1 in this study. Thus, EC50 of LTB on ELISA plate coated with **14** was determined to be 31.6 nM, while on the plate coated with bovine GM1 it was 70.7 nM. The undec-10-enyl GM2 mimic **11** showed minor activity, while the GM3 analogue **8** as expected was practically inactive, thus giving a confirmation of the specificity of binding.

Subsequently, we tested the binding of the GM1 mimic **14** to the B subunit of cholera toxin and three monoclonal antibodies (mAbs) to GM1, specifically mouse IgG mAbs DG1 and DG2 and human IgM mAbs SM1. The cholera toxin B-subunit and all monoclonal antibodies displayed a similar binding to the

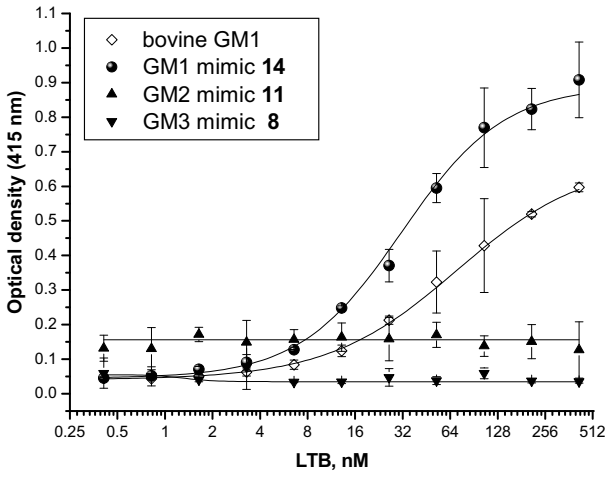


Figure 2. ELISA titration of the binding of B subunit of *E. coli* heat-labile enterotoxin (LT-B) to natural and synthetic gangliosides coated on ELISA plates, as visualized using a monoclonal antibody specific for LT-B-pentamer.

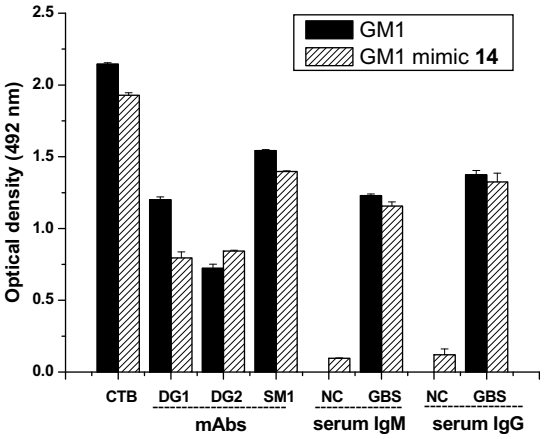


Figure 3. Binding of cholera toxin B subunit and antibodies to bovine GM1 and GM1 mimic **14**.

natural and synthetic (**14**) GM1 gangliosides coated on ELISA plates (Fig. 3). Moreover, both IgM and IgG antibodies from the sera of patients with GBS recognized the synthetic GM1 analogue **14** to a similar extent as the bovine brain-derived GM1. Serum IgM and IgG from normal controls (NC) showed no activity to this GM1 mimic, demonstrating that its structure does not aspecifically bind to immunoglobulins.

As a result it is clear that GM1 analogue **14** can be used to detect both toxins and antibodies involved in human diseases. Since the structure of this compound is the result of stepwise syntheses, each of which can be controlled and modified using different building blocks, these results encourage studies of the potential of GM1 mimics to supersede the bovine brain-derived GM1 in diagnostic and therapeutic studies. Specifically, such mimics can be useful when these can be applied in counteracting the physiologically detrimental effect of several toxin- or antibody-mediated diseases, and such studies are currently underway in our laboratories.

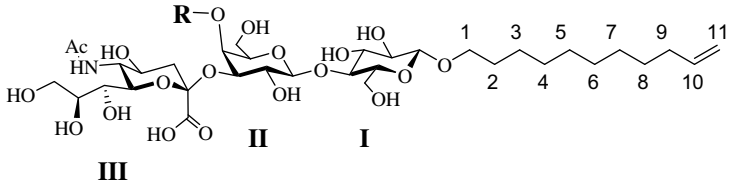
2.3. Assignment of ^{1}H and ^{13}C chemical shifts in 900 MHz NMR spectra of undec-10-enyl glycoside mimics of GM3 (8), GM2 (11) and GM1 (14)

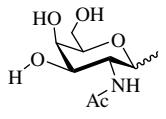
The sugar units in **8**, **11** and **14** are denoted by the pyranoside units '**I**' through '**V**' starting from the aglycone end as shown in Figure 4. As the starting point, all the H–C correlations are established using HSQC spectra.

The olefinic proton resonance at 5.8 ppm can be unambiguously assigned to proton 10 of the tail. The resonances for the protons in the alkyl tail up to H-3 are established in DQF-COSY spectrum, and 2D-TOCSY allows for the assignment of H-2 and H-1 protons. All these resonances in aglycone are confirmed by HSQC and HMBC spectra.

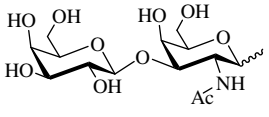
2.3.1. Assignments of ^{1}H and ^{13}C resonances of the glucose (I) residue. Subsequently, the ^{1}H NMR spectrum of GM3 mimic **8** shows two resolved anomeric resonances at 4.46 and 4.31 ppm. Of these, the signal at 4.31 ppm is readily assigned to H-1 of Glc(I), as it shows the cross peak with carbon atom C-1 of the aglycone tail in the HMBC spectrum. Starting with this anomeric resonance, the peaks for the first glucose ring to (I)H-6*b* are assigned in the DQF-COSY spectra as shown in Figure 5.

In the 2D-TOCSY spectrum (Fig. 6), the through-bond connectivity between (I)H-1 and other protons in ring I can be monitored, and the accurate chemical shifts for these hydrogen atoms can be established. The HMBC spectrum of GM3 mimic **8** shows the (II)C-1/





GM3 mimic 8: **R** = H; **GM2 mimic 11**: **R** =



GM1 mimic 14: **R** =

Figure 4. Compounds under study, and numbering of C atoms (1–11) and saccharide rings (**I**–**V**).

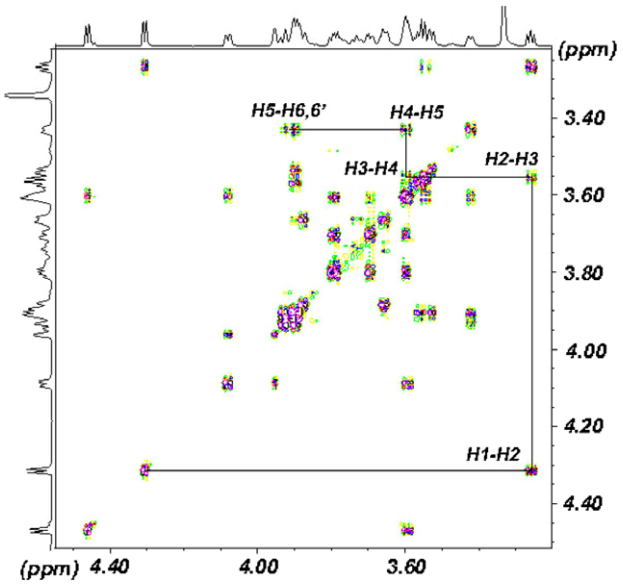


Figure 5. DQF-COSY spectrum of GM3 mimic **8**. Solid lines show through-bond connectivities of the glucose ring.

(I)H-4 coupling peak across the glycosidic bond, which confirms the assignment of the (I)H-4 resonance. Resonances of (I)H-6*a* and (I)H-6*b* are accurately assigned by (I)C-6/(I)H-6*a* and (I)C-6/(I)H-6*b* cross peaks in the HSQC spectrum. Once the chemical shifts of the H atoms are established, the assignments for the C atoms can be obtained using HSQC.

2.3.2. Assignments of ^{1}H and ^{13}C resonances of the galactose (II) residue. The doublet at 4.45 ppm (in **11** and **14**) or 4.46 ppm (in **8**) can be assigned to (II)H-1, as the signal obtained from the HSQC spectrum of the corresponding carbon atom shows a clear correlation with (I)H-4 in the HMBC spectrum (Fig. 7).

In the TOCSY spectrum of **8** (Fig. 8), the spin–spin information transfer from (II)H-1 to (II)H-2, (II)H-3 and (II)H-4 is clearly seen. The chemical shifts of (II)H-5 and (II)H-6 protons are obtained from both 2D-TOCSY and HMBC spectra. In the TOCSY spectrum, through-bond connectivity for the (II)H-4 signal is not only observed to the (II)H-1, (II)H-2 and (II)H-3

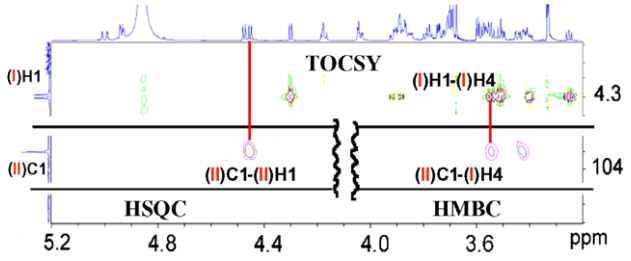


Figure 7. Parts of 2D-TOCSY, HSQC and HMBC spectra of GM3 mimic **8**. Red lines show C(1)–H(1) correlation for the galactose (II) ring (left) and correlation between the anomeric carbon atom of the galactose ring with the glucose H-4 proton (right).

signals, but also to two additional signals at 3.70 ppm and 3.89 ppm. Of these, the signal at 3.7 ppm has a cross-peak correlated with the anomeric (II)C-1 carbon, and is thus assigned to (II)H-5. The other signal is then assigned to (II)H-6. The ^{13}C chemical shifts are subsequently obtainable from the HSQC spectrum. For **11** and **14**, the ^{13}C resonance of the (II)C-4 atom is shifted 10 ppm downfield, which supports the proposed linkage site of rings II and IV.

2.3.3. Assignments of ^{1}H and ^{13}C chemical shifts of the NeuAc (III) residue. In the TOCSY spectra of GM3 mimic **8**, GM2 mimic **11** and GM1 mimic **14**, spin–spin information transfer from (III)H-3$_{eq}$ to (III)H-4, (III)H-5, (III)H-6 and (III)H-7 is observed (Fig. 9; example given for **14**). The assignments can be unambiguously made by cross peaks in DQF-COSY (for H-4) and HSQC (for H-5) spectra.

For the (III)H-7 signal, through-bond connectivity to the (III)H-8 and (III)H-9 protons is observed in these TOCSY spectra, and HSQC allows for the assignment thereof (Fig. 10).

2.3.4. Assignments of ^{1}H and ^{13}C chemical shifts of the GalNAc (IV) residue. Of all the anomeric hydrogen resonances, the lowest field signal in the ^{1}H spectrum of **11** is assigned to (IV)H-1 of the GalNAc (IV) residue, on the basis of the HMBC spectrum of **11** that shows the (IV)C-1\(II)H-4 cross peak across the glycosidic bond.

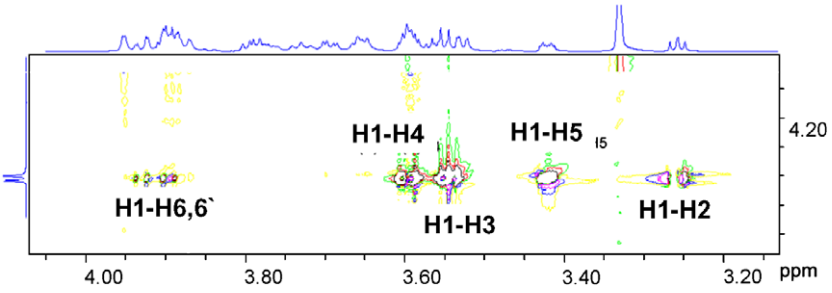


Figure 6. Part of the 2D-TOCSY spectrum of GM3 mimic **8** showing through-bond connectivities of ring (I).

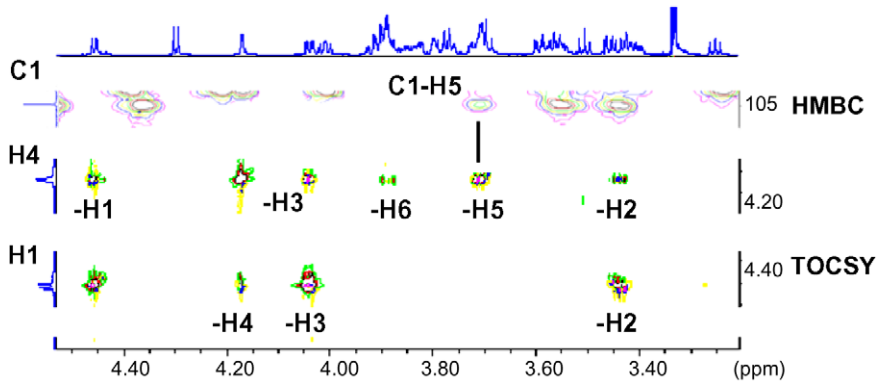


Figure 8. Parts of HMBC and TOCSY spectra of GM3 mimic **8** for the galactose (II) ring. The black line shows correlation between the anomeric C atom and one of the protons, which was thus assigned to (II)H-5.

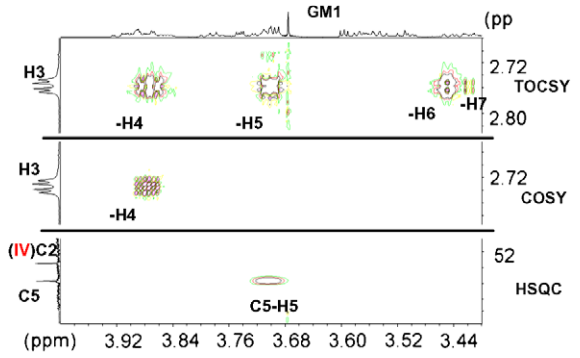


Figure 9. Partial TOCSY, DQF-COSY and HSQC spectra for the NeuAc (III) residue in GM1 mimic **14**.

Cross peak connectivities in the DQF-COSY spectrum subsequently yield proton assignments for the H-2, H-3 and H-4 resonances of ring IV (Fig. 11).

The chemical shifts of (IV)H-5 and (IV)H-6 are determined analogously to those of the ring II by the TOCSY and HSQC spectra. Thus, the TOCSY spectrum of **11** shows magnetization transfer from (IV)H-4 to signals at 3.83 and 3.78 ppm (Fig. 12), and the cross peak with the methylene carbon signal in HSQC spectrum allows the assignment of the H-6 resonances (Fig. 13). The carbon chemical shifts are subsequently obtainable from the HSQC spectrum. For GM1 mimic **14**, the ^{13}C resonance of the (IV)C-3 atom is shifted 9 ppm downfield with respect to its value in **11**, which thus provides additional confirmation for the linkage site of rings IV and V.

2.3.5. Assignments of ^{1}H and ^{13}C chemical shifts of the galactose (V) residue. Finally, the remaining anomeric hydrogen signal at 4.47 ppm in the spectrum of GM1 mimic **14** is assigned to (V)H-1 of the terminal galactose residue. The remaining ^{1}H chemical shifts can then be assigned from this anomeric signal using DQF-COSY and HSQC. Similar to the approach used for the II and IV sugar units, the chemical shifts for H atoms up to (V)H-4 can be established using DQF-COSY, in the

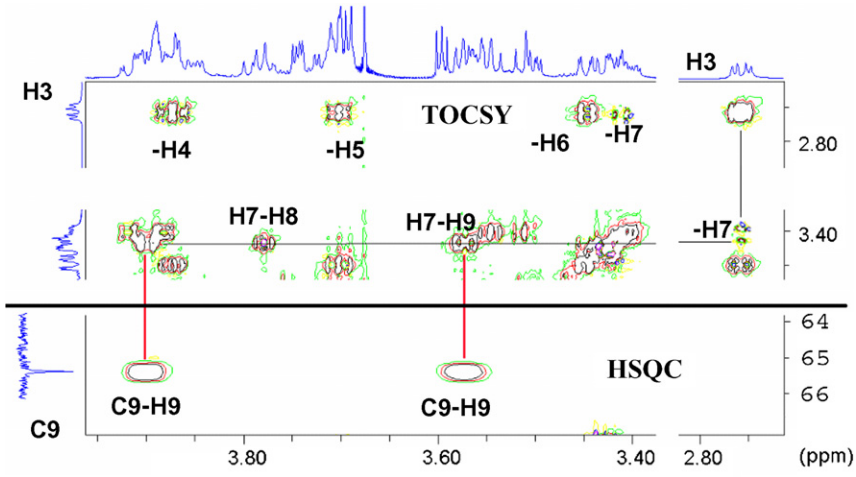


Figure 10. Partial TOCSY and HSQC spectra of GM1 mimic **14**. The black line shows through-bond connectivities for the (III)H-7 proton of the NeuAc ring, while red lines indicate C–H correlations of the (III)C-9 carbon with the two (III)H-9 protons.

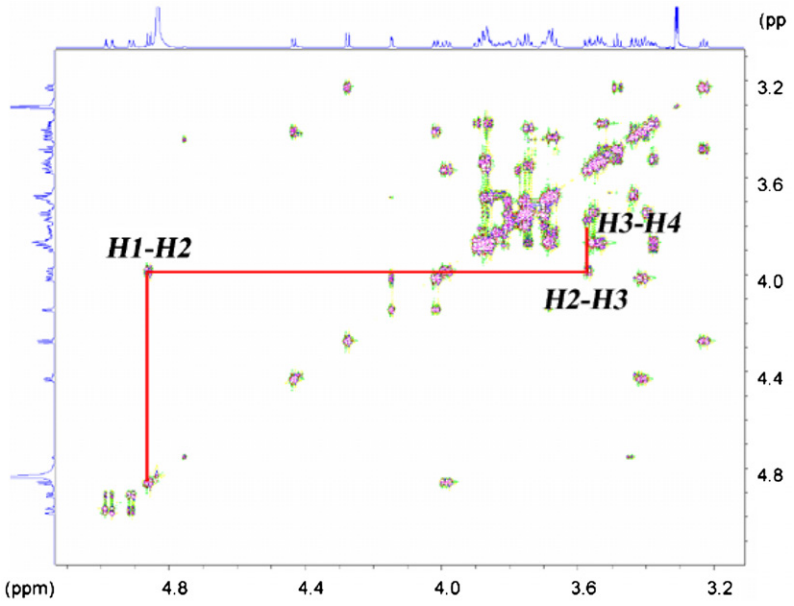


Figure 11. DQF-COSY spectrum of GM2 mimic **11**. Red lines show through-bond connectivity of the GalNAc (IV) ring.

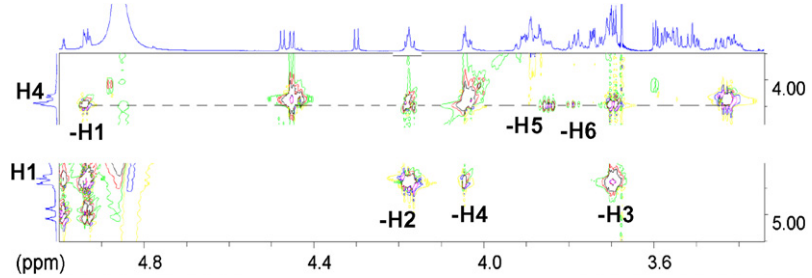


Figure 12. Part of the TOCSY spectrum of GM2 mimic **11** showing correlations for the H-1 and H-4 protons of saccharide ring IV.

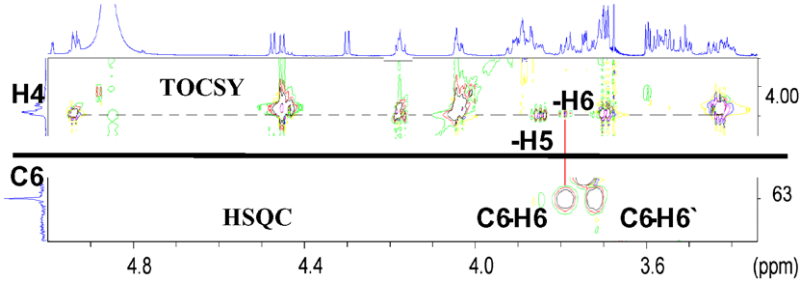


Figure 13. Partial TOCSY and HSQC spectra of GM2 mimic **11**. The red line shows the assignment of one of the H-6 protons of ring IV based on C–H correlations.

case of **14** (Fig. 14). The only unassigned CH_2OH carbon signal can be assigned to the (V)C-6 atom of ring V, and the (V)H-6 chemical shifts are established from HSQC cross peaks. The (V)H-5 chemical shift can then finally be established by the cross peak H-6\H-5 in the DQF-COSY spectrum (Fig. 15).

The complete assignment of proton and carbon resonances for the ganglioside mimics **8**, **11** and **14** is summarized in Tables 1 and 2. Signals of aglycone tails of these compounds are presented in Section 4.

3. Conclusion

We have successfully extended a chemoenzymatic method for the efficient synthesis of glycan chains of

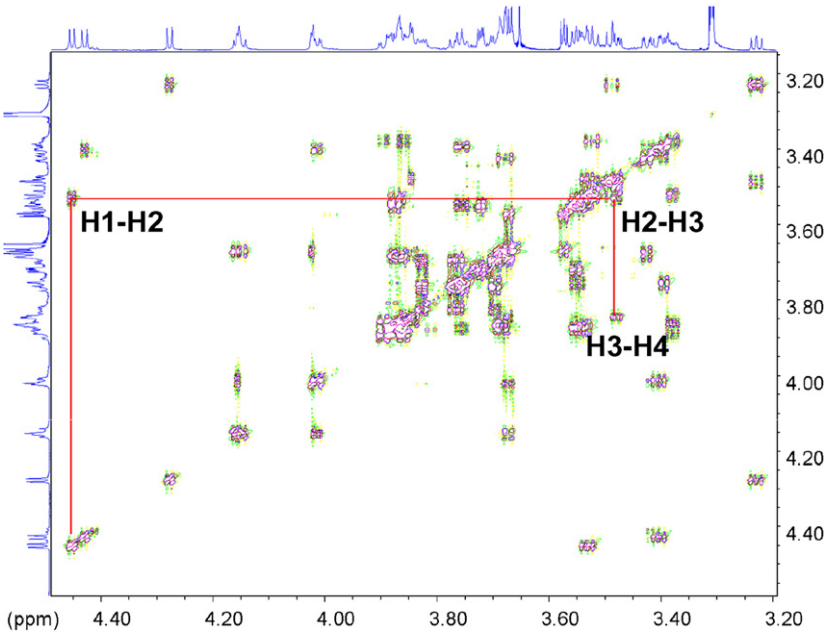


Figure 14. DQF-COSY spectrum of GM1 mimic **14**. Red lines show through-bond connectivity of the terminal galactose ring.

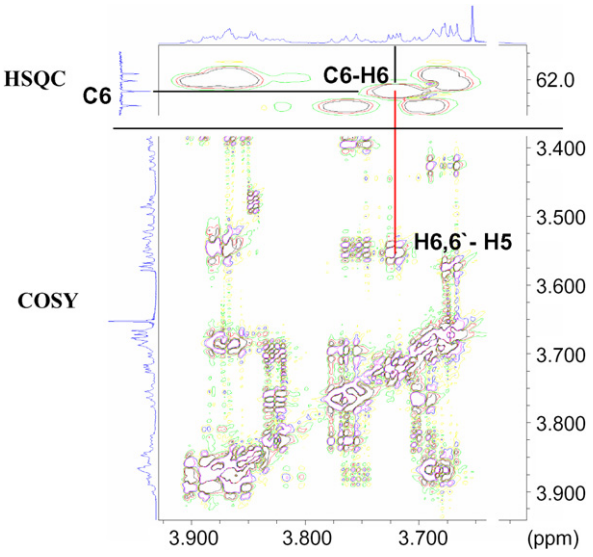


Figure 15. Partial HSQC and DQF-COSY spectra of GM1 mimic **14**.

gangliosides[52] to poorly water-soluble lactosides, and showed the latter to be excellent substrates for *C. jejuni* glycosyltranferases. As such an extension to methanol/water mixtures may be rather general, this will simplify the synthesis of a wide variety of oligosaccharides that are of interest to be attached onto solid substrates for sensing or diagnostics purposes, as in carbohydrate arrays.[65,66] A full ab initio assignment was made of all ^{1}H and ^{13}C chemical shifts in the 900 MHz NMR spectra of the synthesized compounds GM3, GM2 and GM1 derivatives **8**, **11** and **14**. This approach will also allow for the full assignment of the sugar moieties of other (naturally occurring) glycolipids.

Binding studies of GM1 analogue **14** with bacterial toxins and antibodies from the sera of GBS patients clearly demonstrated that such GM1 mimics can be efficiently used to detect both toxins and antibodies involved in human diseases. This opens up their application as valuable alternatives for bovine brain-derived GM1 in diagnostic and therapeutic studies.

4. Experimental

4.1. General methods

NMR spectra were recorded on a Bruker Avance 900 spectrometer. All the measurements were done at 298 K in CD_3OD. HRMS data were collected on a Q-TOF Ultima (Waters Corporation) machine. Solvents and chemicals were purchased from Sigma Chemical Co. Thin layer chromatography (TLC) was performed on Merck Silica Gel 60F254 aluminium backed plates, and detection was realized by either of the following methods: UV (254 nm), charring with a solution of $KMnO_4$ (aq) or with 5% (v/v) sulfuric acid in ethanol and subsequent heating.

4.2. Enzymes

The *C. jejuni* UDP-GlcNAc 4-epimerase (construct CPG-13) was expressed and purified as described by Bernatchez et al.[67] The *C. jejuni* Cst-I α-(2,3)-sialyltrans-

Table 1. Assignment of ^{13}C resonances in GM3 mimic **8**, GM2 mimic **11** and GM1 mimic **14**

Sugar unit	Atom number	Chemical shift, ppm		
		GM3 mimic **8**	GM2 mimic **11**	GM1 mimic **14**
βDGlc	1	104.25	104.21	104.19
	2	74.75	74.77	74.75
	3	76.43	76.31[a]	76.31[a]
	4	81.01	81.40	81.44
	5	76.38	76.39	76.38
	6	62.04	62.01	62.01
βDGal	1	105.06	104.88	104.91
	2	70.86	71.08	71.07
	3	77.51	76.40[a]	76.34[a]
	4	69.13	79.02	79.02
	5	76.87	75.57	75.58
	6	62.54	61.75	61.74
αDNeuAc	1	175.41	175.66	175.67
	2	101.07	103.42	103.45
	3	41.83	38.61	38.57
	4	69.26	69.64[a]	69.67[a]
	5	54.13	53.80	53.79
	6	74.82	75.10	75.09
	7	70.08	70.41	70.43
	8	73.00	73.39	73.38
	9	64.48	65.33	65.34
	N–C=O	175.50	175.11	175.21
	CH_3	22.66	22.60	22.61
βDGalNAc	1		104.24	104.09
	2		54.23	52.70
	3		74.07	82.96
	4		69.84[a]	69.72[a]
	5		76.26	75.89
	6		63.00	62.96
	N–C=O		174.74	174.84
	CH_3		23.60	23.77
βDGal	1			106.57
	2			72.50
	3			74.58
	4			70.24
	5			76.48
	6			62.40

[a] Examples of assignments that could not be determined at lower fields.

Table 2. Assignment of ^{1}H resonances in GM3 mimic **8**, GM2 mimic **11** and GM1 mimic **14**

Sugar unit	Atom number	Chemical shift		
		GM3 mimic **8**	GM2 mimic **11**	GM1 mimic **14**
βDGlc	1	4.305	4.297	4.301
	2	3.258	3.251	3.253
	3	3.545	3.505	3.513
	4	3.595	3.547	3.544
	5	3.421	3.400	3.402
	6_b	3.924	3.912	3.913
	6_a	3.902	3.889	3.887
βDGal	1	4.459	4.453	4.451
	2	3.593	3.429	3.431
	3	4.079	4.037	4.036
	4	3.953	4.168	4.178
	5	3.596	3.704	3.708
	6_b	3.792	3.888	3.891
	6_a	3.694	3.704	3.709
αDNeuAc	3_{eq}	2.889	2.760	2.758
	3_{ax}	1.778	1.925	1.929
	4	3.773	3.850	3.872
	5	3.732	3.695	3.701
	6	3.656	3.457	3.450
	7	3.527	3.419	3.417
	8	3.897	3.766	3.777
	9_b	3.874	3.892	3.899
	9_a	3.654	3.572	3.570
	CH_3	2.044	2.036	2.040
βDGalNAc	1		4.858	4.938
	2		4.009	4.176
	3		3.592	3.696
	4		3.797	4.046
	5		3.825	3.849
	6_b		3.777	3.788
	6_a		3.717	3.722
	CH_3		2.036	2.017
βDGal	1			4.474
	2			3.551
	3			3.503
	4			3.868
	5			3.576
	6_b			3.744
	6_a			3.744

ferase (construct CST-06) was expressed as a fusion protein with the *E. coli* maltose-binding protein (without the leader peptide) and purified on amylose resin according to the manufacturer's instructions (New England Biolabs, Beverly, MA). The *C. jejuni* β-(1,3)-galactosyltransferase (construct CJL-20) was expressed and purified as described by Linton et al.[68] The *C. jejuni* β-(1,4)-*N*-acetylgalactosaminyltransferase (construct CJL-30) was expressed as described by Blixt et al.[52] The cell extracts were prepared using an Avestin C5 Emulsiflex cell disruptor (Avestin, Ottawa, Canada). In the case of CJL-30, we did not purify the enzyme but used an extract that was clarified by centrifugation at 27,000 *g* and stored at −20 °C in 50 mM Hepes buffer (pH 7) with 40% glycerol.

4.3. Undec-10-enyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (1)

Undec-10-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6**) (1 g) was dissolved in anhydrous CH_3OH (10 mL). A freshly prepared 1 M NaOMe solution in anhydrous CH_3OH (100 μL) was added, and the mixture was stirred overnight at room temperature. More methanol (50 mL) was added to dissolve the formed precipitate, followed by the addition of the acidic ion exchange resin Amberlite IR-120 H, and the mixture was stirred for 10 min. The resin was filtered off, washed with

methanol and the filtrate concentrated under reduced pressure to give 0.62 g (99%) of **1** as a white powder which was used without additional purification. Selected ^{1}H NMR (400 MHz, CD_3OD): δ 5.87–5.74 (m, 1H, –CH_2–C*H*=CH_2), 5.04–4.91 (m, 2H, –CH_2–CH=C*H*$_2$), 4.21 (d, 1H, *J* 7 Hz, H_{Gal}-1), 4.17 (d, 1H, *J* 7.5 Hz, H_{Gluc}-1), 3.01 (m, 1H, H_{Gluc}-2), 2.06–1.97 (m, 2H, –C*H*$_2$–CH=CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 139.71, 115.41, 104.78, 103.40, 81.71, 79.98, 76.43, 75.86, 75.62, 74.12, 74.00, 71.46, 69.58, 68.91, 61.44, 61.20, 34.03, 31.47, 29.85, 29.75, 29.69, 29.37, 29.13.

4.4. Undec-10-ynyl 2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (7)

A solution of octa-*O*-acetyl-β-lactose (**5**) (3.00 g, 4.42 mmol), undec-10-yn-1-ol (1.49 g, 8.84 mmol) and 4Å (1.0 g) molecular sieves in CH_2Cl_2 (anhydrous, 30 mL) was stirred under nitrogen at room temperature for 30 min and then cooled on ice. Subsequently, boron trifluoride etherate (5 mL, 35.37 mmol) was added dropwise over a period of 5 min and the whole was stirred under nitrogen atmosphere overnight while it was slowly reaching room temperature. The reaction mixture was poured onto ice water (50 mL) with stirring. The organic layer was separated, washed successively with 10% aqueous $KHCO_3$ and brine and dried over sodium sulfate. The solvent was evaporated in vacuo to give 4.89 g of residue. Column chromatography (gradient of EtOAc/PE = 1:2 to 100% EtOAc) yielded **7** (1.37 g, 40%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.18 (dd, 1H, $J_{3',4'}$ 3.4, $J_{4',5'}$ 0.8 Hz, $H_{4'}$), 5.03 (t, 1H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H_3), 4.93 (dd, 1H, $J_{2',3'}$ 10.5, $J_{1',2'}$ 7.9 Hz, $H_{2'}$), 4.82 (dd, 1H, $J_{2',3'}$ 10.5 $J_{3',4'}$ 3.4 Hz, $H_{3'}$), 4.72 (dd, 1H, $J_{2,3}$ 9.0 $J_{1,2}$ 7.5 Hz, H_2), 4.38 (d, 1H, $J_{1',2'}$ 7.9 Hz, $H_{1'}$), 4.32 (d, 1H, $J_{1,2}$ 7.5 Hz, H_1), 4.31 (m, 1H, H_{6a}), 4.01–3.90 (m, 3H, H_{6b}, $H_{6'a}$, $H_{6'b}$), 3.79 (m, 1H, $H_{5'}$), 3.68 (m, 1H, –OCH_a–), 3.66 (~t, 1H, $J_{4,5}$ 9.5, $J_{3,4}$ 9.0 Hz, H_4), 3.47 (ddd, 1H, $J_{4,5}$9.5, $J_{5,6b}$ 5.5, $J_{5,6a}$ 2.0 Hz, H_5), 3.3 (dt, 1H, ^2J 9.5, ^3J 6.5 Hz, –OCH_b–), 2.04–1.98 (m, 5H, –C*H*$_2$–C≡CH, Ac at 1.99), 1.96 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.90–1.88 (m, 6H, 2 × Ac), 1.87 (s, 3H, Ac), 1.83 (t, 1H, –CH_2–C≡C*H*), 1.8 (s, 3H, Ac), 1.47–1.30 (m, 4H, –$(CH_2)_2$–), 1.27–1.08 (m, 10H, –$(CH_2)_5$–). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 169.9, 169.8, 169.6, 169.4, 169.1, 168.7 (7 × C=O), 100.6 ($C_{1'}$), 100.2 (C_1), 84.3 (–CH_2–*C*≡CH), 76.0 (C_4), 72.5 (C_3), 72.2 (C_5), 71.3 (C_2), 70.6 ($C_{3'}$), 70.2 ($C_{5'}$), 69.7 (–OCH_2–), 68.8 ($C_{2'}$), 68.0 (–CH_2–C≡*C*H), 66.4 ($C_{4'}$), 61.8 (C_6), 60.6 ($C_{6'}$), 29.0, 28.9, 28.8, 28.6, 28.3, 28.0, 25.4 (–$(CH_2)_7$–), 20.4, 20.3, 20.2, 20.1 (7 × *C*H_3–C(O)–, peaks overlap), 18.0 (–*C*H_2–C≡CH). QTOF-MS $[M+H]^+$ 787.3411 (calcd 787.3388).

4.5. Undec-10-ynyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (2)

Compound **7** (1.37 g) was dissolved in anhydrous CH_3OH (15 mL) and 150 μL of freshly prepared 1M sodium methanolate in anhydrous CH_3OH was added to the solution. The mixture was heated to reflux for 15 min. After cooling, more methanol was added to dissolve the precipitate, followed by neutralization with Amberlite IR-120 H for 10 min. The resin was filtered off, washed with methanol and the filtrate concentrated under reduced pressure to give 0.91 g (100%) of **2** as a white powder which was used without further purification. Selected ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.20 (d, 1H, *J* 7 Hz, H_{Gal}-1), 4.15 (d, 1H, *J* 8 Hz, H_{Gluc}-1), 3.00 (m, 1H, H_{Gluc}-2), 2.65 (t, 1H, *J* 2.5 Hz, –CH_2–C≡C*H*), 2.14 (td, 2H, *J* 6.5 Hz, *J* 2.5 Hz, –C*H*$_2$–C≡CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 104.22, 103.02, 84.98, 81.19, 75.98, 75.48, 75.24, 73.74, 73.64, 71.28, 71.07, 69.13, 68.61, 61.12, 60.86, 29.67, 29.27, 29.20, 28.81, 28.50, 28.35, 25.87, 18.08.

4.6. Undec-10-enyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (8)

Compound **1** (100 mg, 202.4 μmol) was first dissolved in 10 mL of methanol. We then added 30 mL of CMP-Neu5Ac (200 mg, 325.5 μmol, dissolved in water), 2.5 mL of 1 M Hepes buffer (pH 7.5), 0.5 mL of 1 M $MgCl_2$ and 2.5 mL (14 U) of CST-06. The reaction was kept at 37 °C for 1 h, after which 100% of the starting material was converted to product. The reaction was repeated once to convert a total of 200 mg of compound **1** to compound **8**. One third of the reaction mixture was centrifuged and loaded on a Sep-Pak column equilibrated with methanol. Hydrophilic material was washed off with water and the product was eluted with methanol. Appropriate fractions were collected and evaporated to give 84 mg (92%) of compound **8** as white powder. $[\alpha]_D$ +2 (*c* 0.5, H_2O). QTOF-MS $[M+H]^+$ 786.3794 (calcd 786.3755, Δ ppm = 5.0).

4.7. Undec-10-enyl (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)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (11)

60 mL of the previous reaction mixture containing approximately 212 mg (270 μmol) of GM3 oligosaccharide **8** was used directly for the synthesis of the GM2 oligosaccharide (**11**). We added 50 mL of UDP-GlcNAc (260 mg, 399 μmol, dissolved in water), 1.2 mL of 1 M $MnCl_2$, 5 mL (23 U) of CJL-30 and 3 mL (22.5 U) of CPG-13. The reaction was kept at 37 °C for 2 h. At that point at least 95% of the starting material was converted

to product and the reaction mixture was centrifuged and loaded on a Sep-Pak column equilibrated with methanol. Hydrophilic material was washed off with water and the product was eluted with methanol. Appropriate fractions were collected and evaporated to give 265 mg (268 μmol, 99.3% yield) of compound **11** as white powder. $[\alpha]_D$ +12 (*c* 0.34, H_2O). QTOF-MS $[M+H]^+$ 989.4555 (calcd 989.4549, Δ ppm = 0.6).

4.8. General procedure for the synthesis of GM2 analogues 12 and 13

Lactoside **2** or **3** (300 μmol) was dissolved in methanol (20 mL). Subsequently, aqueous solution of CMP-Neu5Ac (500 μmol), Hepes buffer (1 M, 4 mL, pH 7.5), $MgCl_2$ (1 M, 0.8 mL) and CST-06 (10.5 U) were added. The total volume of the reaction mixture was brought to 80 mL with water. The reaction was kept at 37 °C for 1 h, after which 100% of the starting material was converted to product (TLC analysis). The reaction was repeated to convert a total of 750 mg of compound **2** and 500 mg of compound **3**. The combined reaction mixtures of each compound were centrifuged and the supernatant was used directly for the synthesis of GM2 oligosaccharide.

To 100 mL of thus prepared solution of GM3 oligosaccharide **9** or **10**, aqueous solutions of UDP-GlcNAc (50 mM, 10 mL), $MnCl_2$ (1 M, 2 mL), CJL-30 (6.2 U/mL, 15 mL) and CPG-13 (193.6 U/mL, 4 mL) were added. The total volume of the reaction mixture was brought to 200 mL with water and the reaction was kept at 37 °C for 2 h. The reaction mixture was centrifuged and loaded on a Sep-Pak column equilibrated with methanol. Hydrophilic material was washed off with water and the product was eluted with methanol. Appropriate fractions, containing the product, were collected and evaporated to give compound **12** or **13** quantitatively.

4.8.1. Undec-10-ynyl (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)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (12).

$[\alpha]_D$ +17 (*c* 0.59, H_2O). Selected ^1H NMR (400 MHz, CD_3OD): δδ 4.78 (d, 1H, *J* 8.6 Hz, H_{GalNAc}-1), 4.43 (d, 1H, *J* 7.8 Hz, H_{Gal}-1), 4.24 (d, 1H, *J* 7.8 Hz, H_{Gluc}-1), 3.23 (m, 1H, H_{Gluc}-2), 2.66 (dd, 1H, *J* 4 Hz, H_{NeuAc}-3eq). ^{13}C NMR (100 MHz, CD_3OD): δ 175.39, 175.00, 174.50, 104.20 (C_{Gal}-1), 103.77 (C_{Gluc}-1), 103.75 (C_{GalNAc}-1), 102.82 (C_{NeuAc}-2), 84.63 (CH_2–*C*≡CH), 80.35 (C_{Gluc}-4), 78.48 (C_{Gal}-4), 75.96 (C_{Gluc}-5), 75.96 (C_{Gal}-3), 75.87 (C_{GalNAc}-5), 75.57 (C_{Gluc}-3), 75.17 (C_{Gal}-5), 74.58 (C_{NeuAc}-6), 74.36 (C_{Gluc}-2), 73.20 (C_{GalNAc}-3), 73.15 (C_{NeuAc}-8), 70.91 (C_{Gal}-2), 70.37 (*C*H_2-OC_{Gluc}1), 69.97 (C_{NeuAc}-7), 69.23 (C_{GalNAc}-4), 69.21 (C_{NeuAc}-4), 68.31 (CH_2–C≡*C*H), 64.70 (C_{NeuAc}-9), 62.55 (C_{GalNAc}-6), 61.64 (C_{Gluc}-6), 61.41 (C_{Gal}-6), 54.03 (C_{GalNAc}-2), 53.61 (C_{NeuAc}-5), 38.77 (C_{NeuAc}-3), 29.78, 29.00, 28.72, 28.66, 28.41, 28.18, 25.65, 22.83 (C_{GalNAc}–CH3), 22.49 (C_{NeuAc}–CH3), 18.10 (–*C*H_2–C≡CH).

4.8.2. 10-Azidoundecyl (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)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (13).

$[\alpha]_D$ +15 (*c* 0.3, H_2O). Selected ^1H NMR (400 MHz, CD_3OD): δ 4.79 (d, 1H, *J* 8.6 Hz, H_{GalNAc}-1), 4.42 (d, 1H, *J* 7.8 Hz, H_{Gal}-1), 4.26 (d, 1H, *J* 7.8 Hz, H_{Gluc}-1), 3.27 (t, 2H, *J* 6.8 Hz, CH_2-N_3), 3.22 (m, 1H, H_{Gluc}-2), 2.66 (dd, 1H, *J* 4 Hz, H_{NeuAc}-3eq). ^{13}C NMR (100 MHz, CD_3OD): δ 175.47, 105.11 (C_{Gal}-1), 104.26 (C_{GalNAc}-1), 104.11 (C_{Gluc}-1), 81.46 (C_{Gluc}-4), 78.64 (C_{Gal}-4), 76.84 (C_{Gal}-3), 76.61 (C_{Gluc}-5), 76.42 (C_{Gluc}-3), 76.39 (C_{GalNAc}-5), 75.64 (C_{Gal}-5), 75.33 (C_{NeuAc}-6), 74.79 (C_{Gluc}-2), 74.49 (C_{GalNAc}-3), 73 (C_{NeuAc}-8), 70.98 (*C*H_2-OC_{Gluc}1), 70.95 (C_{Gal}-2), 70.44 (C_{NeuAc}-7), 69.79 (C_{GalNAc}-4), 69.15 (C_{NeuAc}-4), 65.43 (C_{NeuAc}-9), 62.97 (C_{GalNAc}-6), 62.03 (C_{Gluc}-6), 61.65 (C_{Gal}-6), 54.65 (C_{GalNAc}-2), 53.64 (C_{NeuAc}-5), 52.5 (CH_2-N_3), 38.75 (C_{NeuAc}-3), 30.82, 30.73, 30.66, 30.62, 30.31, 29.96, 27.87, 27.13, 23.43 (C_{GalNAc}–CH_3), 22.68 (C_{NeuAc}–CH_3).

4.9. Undec-10-enyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-[(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→3)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (14)

Compound **11** (30 mg, 30.6 μmol) and UDP-Gal (27.5 mg, 45 μmol) were dissolved in 2.4 mL of water. We then added 1 mL of 1 M Mes buffer (pH 6), 0.2 mL of 1 M $MnCl_2$, 0.02 mL of 1 M dithiothreitol and 16 mL of CJL-20 (3.35 units). The reaction was kept at 37 °C for 4 h. The mixture was centrifuged and loaded on a Sep-Pak column equilibrated with methanol. Hydrophilic material was washed off with water and the product was eluted with methanol. Appropriate fractions were collected and evaporated to give 33 mg (28.7 μmol, 93.8% yield) of compound **14** as white powder. $[\alpha]_D$ +7.8 (*c* 0.26, H_2O). QTOF-MS $[M+H]^+$ 1151.5054 (calcd 1151.5067, $\Delta = -1.1$ ppm).

4.10. General procedure for the synthesis of GM1 analogues 15 and 16

Compound **12** or **13** (100 mg, 101 μmol of **12** or 97 μmol of **13**) and UDP-Glc (75 mg, 122 μmol) were dissolved in HEPES buffer (17.6 mL, pH 7.5). Subsequently, aqueous solutions of $MnCl_2$ (1 M, 0.2 mL), dithiothreitol (0.1 M, 0.2 mL), CPG-13 (0.5 mL, 270 U/mL, 135 units) and CJL-137[71] (1.5 mL, 0.5 U/mL, 0.75 units) were added.

The whole was incubated at 37 °C for 2 h. The mixture was centrifuged and loaded on a Sep-Pak column equilibrated with methanol. Hydrophilic material was washed off with water and the product was eluted with methanol. Appropriate fractions were collected and evaporated to give GM1 oligosaccharides **15** and **16**.

4.10.1. Undec-10-ynyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-[(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (15). $[\alpha]_D$ +8.3 (*c* 0.24, H_2O). Selected ^{1}H NMR (400 MHz, CD_3OD): δδ 4.89 (H_{GalNAc}-1), 4.46 (d, 1H, *J* 7.1 Hz, $H_{Gal'}$-1), 4.45 (d, 1H, *J* 7.8 Hz, H_{Gal}-1), 4.27 (d, 1H, *J* 7.8 Hz, H_{Gluc}-1), 3.23 (dd, 1H, $J_{2,3}$ 9.0 $J_{1,2}$ 7.9 Hz, H_{Gluc}-2), 2.74 (dd, 1H, *J* 4 Hz, H_{NeuAc}-3eq). ^{13}C NMR (100 MHz, CD_3OD): δ 175.40, 175.10, 174.60 (C_{NeuAc}-1, C_{NeuAc}–*C*(O)CH_3, C_{GalNAc}–*C*(O)CH_3), 106.09 ($C_{Gal'}$-1), 104.23 (C_{Gal}-1), 103.75 (C_{Gluc}-1), 103.60 (C_{GalNAc}-1), 102.85 (C_{NeuAc}-2), 84.64 (–CH_2–*C*≡CH), 82.09 (C_{GalNAc}-3), 80.39 (C_{Gluc}-4), 78.48 (C_{Gal}-4), 76.10 ($C_{Gal'}$ − 5), 75.95 (C_{Gluc}-5), 75.89 (C_{Gal}-3), 75.57 (C_{Gluc}-3), 75.50 (C_{GalNAc}-5), 75.18 (C_{Gal}-5), 74.57 (C_{NeuAc}-6), 74.34 (C_{Gluc}-2), 74.08 ($C_{Gal'}$-3), 73.14 (C_{NeuAc}-8), 72.04 ($C_{Gal'}$-2), 70.91 (C_{Gal}-2), 70.39 (*C*H_2-OC_{Gluc}1), 69.99 (C_{NeuAc}-7), 69.83 ($C_{Gal'}$-4), 69.24 (C_{NeuAc}-4), 69.11 (C_{GalNAc}-4), 68.31 (–CH_2–C≡*C*H), 64.71 (C_{NeuAc}-9), 62.51 (C_{GalNAc}-6), 62.07 ($C_{Gal'}$-6), 61.64 (C_{Gluc}-6), 61.40 (C_{Gal}-6), 53.60 (C_{NeuAc}-5), 52.50 (C_{GalNAc}-2), 38.73 (C_{NeuAc}-3), 29.34, 29.09, 29.00, 28.71, 28.40, 28.18, 25.64, 23.00 (C_{GalNAc}–CH_3), 22.50 (C_{NeuAc}–CH_3), 18.09 (–*C*H_2–C≡CH).

4.10.2. 10-Azidoundecyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-[(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (16). $[\alpha]_D$ +7.1 (*c* 0.23, H_2O). Selected ^{1}H NMR (400 MHz, CD_3OD): δ 4.88 (H_{GalNAc}-1), 4.46 (d, 1H, *J* 7.1 Hz, $H_{Gal'}$-1), 4.45 (d, 1H, *J* 7.8 Hz, H_{Gal}-1), 4.27 (d, 1H, *J* 7.8 Hz, H_{Gluc}-1), 3.27 (t, 2H, *J* 6.8 Hz, CH_2-N_3), 3.23 (dd, 1H, $J_{2,3}$ 9.0 $J_{1,2}$ 7.9 Hz, H_{Gluc}-2), 2.73 (dd, 1H, *J* 4 Hz, H_{NeuAc}-3eq), 2.02 (s, 3H, NHC(O)CH_3), 2.00 (s, 3H, NHC(O)CH_3), 1.90 (t, 1H, *J* 12 Hz, H_{NeuAc}-3ax). ^{13}C NMR (100 MHz, CD_3OD): δ 175.69, 175.25, 174.99 (C_{NeuAc}-1, C_{NeuAc}–*C*(O)CH_3, C_{GalNAc}–*C*(O)CH_3), 106.61 ($C_{Gal'}$-1), 104.77 (C_{Gal}-1), 104.27 (C_{Gluc}-1), 104.21 (C_{GalNAc}-1), 103.4 (C_{NeuAc}-2), 82.61 (C_{GalNAc}-3), 80.72 (C_{Gluc}-4), 79.12 (C_{Gal}-4), 76.52 ($C_{Gal'}$ − 5), 76.36 (C_{Gluc}-5), 76.33 (2 × C, C_{Gal}-3 and C_{Gluc}-3, peaks overlap), 75.85 (C_{GalNAc}-5), 75.57 (C_{Gal}-5), 75.13 (C_{NeuAc}-6), 74.96 (C_{Gluc}-2), 74.67 ($C_{Gal'}$-3), 73.43 (C_{NeuAc}-8), 72.53 ($C_{Gal'}$-2), 71.25 (C_{Gal}-2), 70.98 (*C*H_2-OC_{Gluc}1), 70.44 (C_{NeuAc}-7), 70.24 ($C_{Gal'}$-4), 69.65 (2 × C, C_{NeuAc}-4 and C_{GalNAc}-4, peaks overlap), 65.39 (C_{NeuAc}-9), 62.97 (C_{GalNAc}-6), 62.42 ($C_{Gal'}$-6), 61.9 (C_{Gluc}-6), 61.85 (C_{Gal}-6), 53.82 (C_{NeuAc}-5), 52.69 (C_{GalNAc}-2), 52.47 (*C*H_2-N_3), 38.61 (C_{NeuAc}-3), 30.8, 30.76, 30.71, 30.65, 30.61, 30.29, 29.94, 27.84, 27.11 (–$(CH_2)_9$–), 23.82 (C_{GalNAc}–C(O)*C*H_3), 22.67 (C_{NeuAc}–C(O)*C*H_3).

4.11. ELISA for the detection of LT-B

Recombinant LT-B was produced in *E. coli* strain PC2923 and purified to homogeneity by affinity chromatography on D-galactose essentially as described.[69] All natural gangliosides were from bovine brain. GM1 and asialo-GM1 were from Sigma Aldrich, St. Louis, USA (G7641, G3018) and lyso-GM1 was from Calbiochem-Novabiochem Corporation, La Jolla, USA (345739).

Microtiter plates (PolySorp Immunoplates, Nunc) were coated with gangliosides at 5 μg/mL in phosphate-buffered saline and serial two-fold dilutions of rec-LT-B were loaded onto coated plates. Binding was measured using an LT-B-specific monoclonal antibody as described.[70]

4.12. ELISA for the detection of CT-B and anti-GM1 antibodies

Bovine brain-derived GM1 (Sigma Aldrich, St. Louis, USA, G7641) (200 pmol/well) and synthetic GM1- analogue **14** (300 pmol/well) were coated on microtiter plates (MaxiSorb Immunoplates, Nunc). Wells were incubated with peroxidase-conjugated cholera toxin B subunit (CT-B), mouse IgG monoclonal antibodies (mAbs) DG1 and DG2 (10 μg/mL), human IgM mAbs SM1 (10 μg/mL) and serum samples (diluted 1:100 in phosphate-buffered saline buffer, pH 7.4) from a normal control (NC) and an anti-GM1 IgM and IgG positive patients with Guillain–Barré syndrome (GBS). CT-B was incubated at room temperature for one hour, and the monoclonal antibody and serum dilutions were incubated at 4 °C overnight. The presence of bound mouse monoclonal antibodies, human monoclonal antibodies and serum IgM and IgG antibodies to GM1 and synthetic GM1 analogue was detected by, respectively, peroxidase-conjugated rabbit anti-mouse IgM, anti-human IgM and anti-human IgG as a second step.[21] All the results are given as mean specific optical densities at 492 nm (mean OD of in duplo coated wells minus mean OD of in duplo blanc wells).

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