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for

Targeting Myelin Associated Glycoprotein (MAG): Bioactive Conformations of Gangliosides Control the Design of Inhibitors

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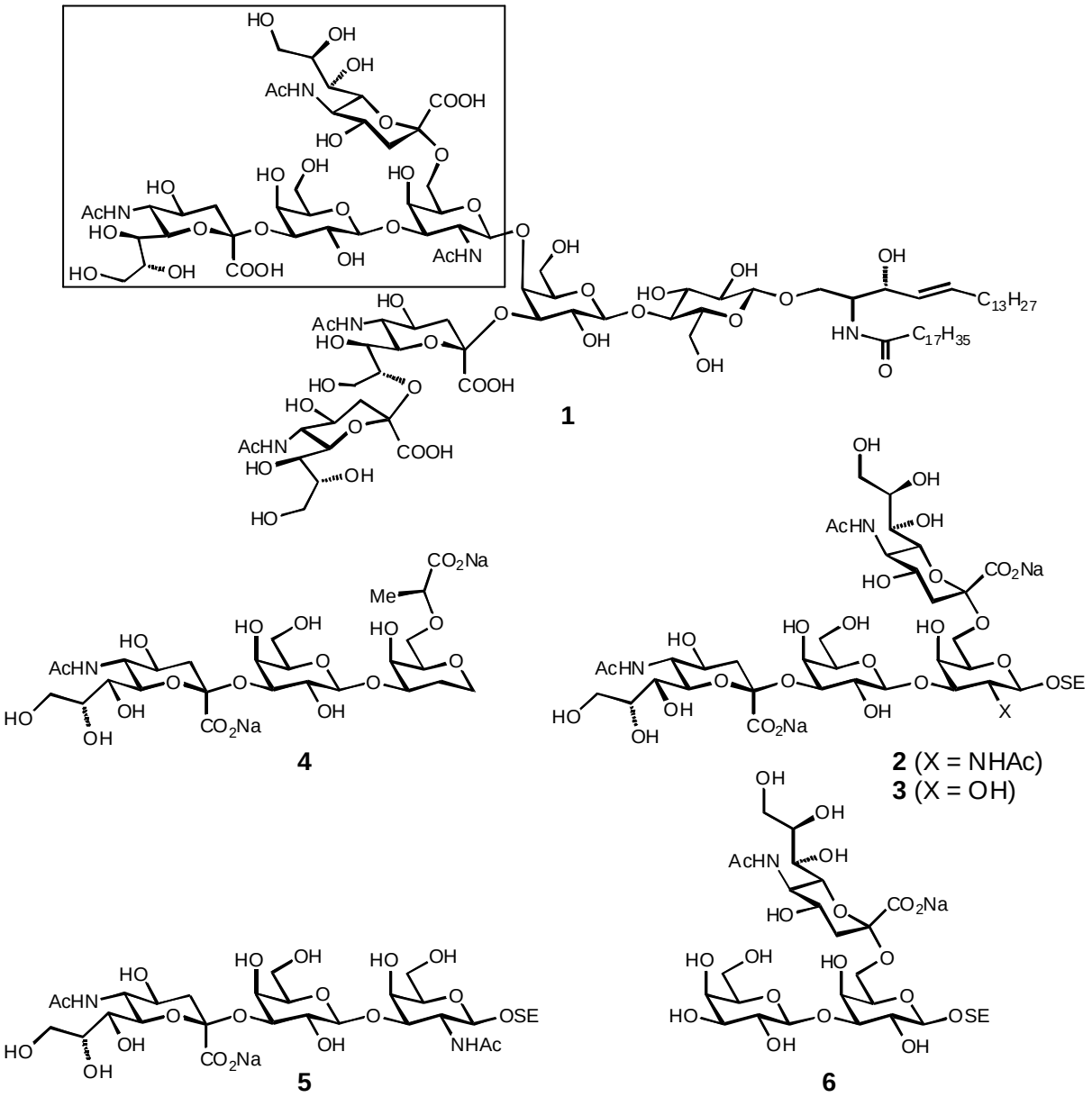
NOESY spectra reflect the solution conformation

The NOESY spectra of the oligosaccharides **2** to **5** are shown in Figure S1. The spectra for saccharides **2** and **5** were obtained at 700 MHz, whereas all other saccharides were measured at 500 MHz. In general, NOEs for the free saccharides were negative at 288 K (tetrasaccharide **3** was measured at 280 K). For the pseudotetrasaccharide **4**, the NOEs were very close to zero at 288 K. From a set of eight NOESY spectra with mixing times between 50 and 750 ms, it was possible to obtain the build-up curves for a number of inter-glycosidic and intra-glycosidic NOEs of trisaccharide **5** and tetrasaccharide **2**. All the investigated saccharides **2** to **5** share a common terminal disaccharide building block Neu5Acα(2→3)Gal. A strong inter-glycosidic NOE between H3 of Gal and H3ax of Neu5Ac was observed in all cases, whereas other inter-glycosidic NOEs: $H3^{Gal}$-$H3_{eq}^{Neu5Ac}$ and $H3^{Gal}$-$H8^{Neu5Ac}$ were weak at this linkage (Figure S1 and Table S1). The central disaccharide unit Galβ(1→3)Gal in **5** and **3**, or Galβ(1→3)Gal*N*Ac in the case of **2**, has much less conformational freedom compared to the Neu5Acα(2→3)Gal linkage. Two prominent inter-glycosidic NOEs were observed between the anomeric proton of the first Gal residue (**5**: H1′; **2** and **3**: H1″, cf.

Scheme S3) and protons H3 and H4 of the second Gal, or the Gal*N*Ac (**5**: H3 and H4; **2** and **3**: H3′ and H4′, cf. Scheme S3). The H1′-H3 inter-glycosidic NOE of trisaccharide **5**, or the H1″-H3′ NOE of tetrasaccharides **2** and **3**, was significantly stronger than the NOE of H1′-H4 for trisaccharide **5** or H1″-H4′ for tetrasaccharides **2** and **3** (Figure S1). Other inter-glycosidic NOEs were of negligible intensities.



Scheme S1.

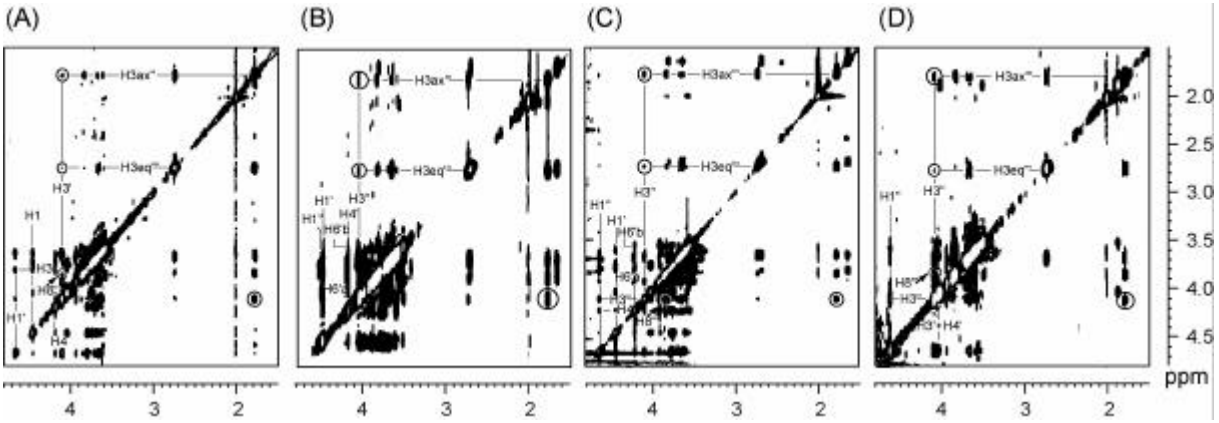


Figure S1. The NOESY spectra of (A) the trisaccharide **5** (700 MHz, 288 K), (B) tetrasaccharide **2** (700 MHz 288 K), (C) tetrasaccharide **3** (500 MHz, 280 K) and (D) pseudo-tetrasaccharide **4** (500 MHz, 288 K). Mixing time of all the NOESY spectrums was 500 ms. In all spectra the strong NOE was observed either between H3′ and H3ax″ (trisaccharide **5**) or between H3″-H3ax‴ (saccharides **2** to **4**). The corresponding H3′-H3eq″ and H3′-H8″ (for trisaccharide **5**) or H3″-H3eq‴ and H3″-H8‴ (for saccharides **2** to **4**) were comparatively smaller. Open circles highlight these cross peaks.

For the Galβ(1→3)-D-Cyc moiety of pseudo-tetrasaccharide **4**, a strong overlap was observed for the H3′ and the H4′ resonances. Therefore, it was not possible to compare the corresponding inter-glycosidic NOEs for H1″-H3′ and H1″-H4′. As all NOEs were very weak due to an unfavorable correlation time of pseudo-tetrasaccharide **4**, the inter-glycosidic NOEs were difficult to observe. A ROESY experiment revealed an additional medium size ROE between H1″ and H2′ (Table S1). The two protons H2eq′ and H2ax′ display the same chemical shift values and therefore, a distinction between the NOEs H1″-H2eq′ and H1″-H2ax′ could not be made (Figure S1). Due to strong signal overlap, it was impossible to unambiguously assign inter-glycosidic NOEs characterizing the Neu5Acα(2→6)Gal*N*Ac building block of tetrasaccharides **2** and **3**. NOE cross peaks between H4′ of Gal or Gal*N*Ac and the two protons at C6' of Gal or Gal*N*Ac were well separated from other signals, even allowing for a proper integration of these peaks but unfortunately the stereo specific assignment of H6proS′ and H6proR′ is not available. For the pseudo-tetrasaccharide **4**, the NOE intensity of the H6′-CH^{Lac} was more intense than that of the H6′-$CH_3{}^{Lac}$ cross peak (Table S1). No other inter-glycosidic cross peaks were observed at the (*S*)-Lac-(2→6)-D-Cyc linkage.

Table S1. Inter-glycosidic NOEs of the saccharides **2** to **5** in 10 mM phosphate buffer (with 150 mM NaCl, pH* 7.4). Relative NOE intensities are defined as: strong (s) (< 2.5 Å), medium (m) (2.5 to 3.6 Å) and weak (w) (> 3.6 Å). (Lac: Lactic acid residue).

Saccharides	Inter glycosidic NOEs		Relative intensity
5 (700 MHz, 288 K)	H3′	H3ax″	s
		H3eq″	w-m
		H8″	w
	H1′	H3	s
		H4	s
2 (700 MHz, 288 K) and **3** (500 MHz, 280 K)	H3″	H3ax‴	s
		H3eq‴	m
		H8‴	w
	H1″	H3′	s
		H4′	m
	H6′	H3ax	w
		H4	w
		H4′	m
4 (500 MHz, 288 K)	H3″	H3ax‴	s
		H3eq‴	w
		H8‴	w
	H1″	(H3′+H4′)	s
		(H2ax′+H2eq′)	m (from ROESY)
	H6′	$-CH^{Lac}$	s
		$-CH_3^{Lac}$	w (from ROESY)

Transfer NOEs reflect bioactive conformations

In the presence of MAG, the ganglioside saccharides **2** to **6** displayed strong negative NOE signals. As all free saccharides show negative NOEs, a clear distinction between NOEs and trNOEs required the acquisition of build-up curves. In the case of pseudo-tetrasaccharide **4**, the distinction was straightforward since NOEs of the free saccharide were very close to zero crossing at 288 K. The build-up curves unambiguously show that trNOEs were observed in all cases. In the presence of MAG, the trNOE patterns were clearly different from the NOE patterns observed for the free saccharides. Transfer NOESY (trNOESY) spectra are shown in Figure S2, and prominent differences are highlighted. Inter-glycosidic trNOEs provide very important information about the conformation of a bound saccharide. Hence, the trNOEs across the α(2→3)-glycosidic linkages were most interesting, since at this linkage the selection of one bioactive conformation was expected upon binding to MAG. In all cases, a strong inter-glycosidic trNOE, H3′-H8″ for trisaccharide **5** or H3″-H8‴ for saccharides **2**, **3** or **4** and a negligibly small H3′-H3ax″ or H3″-H3ax‴ trNOE in the presence of MAG was

found (Figure S2). But a close inspection utilizing NOE/trNOE build-up curve (Figure S3) revealed that the cross peak between H3″ of Gal and H3ax‴ of Neu5Ac was solely due to NOE and not due to trNOE. The maximum trNOE enhancement between H3″-H8‴ was observed at the lower mixing time of ca. 200 ms (Figure S3) whereas for H3″-H3ax‴ cross peak the maximum enhancement was observed at 350 ms mixing time, both in the absence and in the presence of MAG, respectively. Also, the relative intensity of the trNOE between H3″ of Gal and H3ax‴ of Neu5Ac is much smaller than the intra-glycosidic trNOE H3ax‴-H5‴, whereas in aqueous solution of **2** both NOEs H3″-H3ax‴ and H3ax‴-H5‴ were of similar intensity. So, the saccharides **2** to **5** prefer "syn" conformation with $\phi 1, \psi 1 = -60°, -20°$ in the presence of MAG. This result indicates that the $\alpha(2\rightarrow3)$-glycosidic linkage undergoes a major conformational change upon binding to protein (Scheme S2).

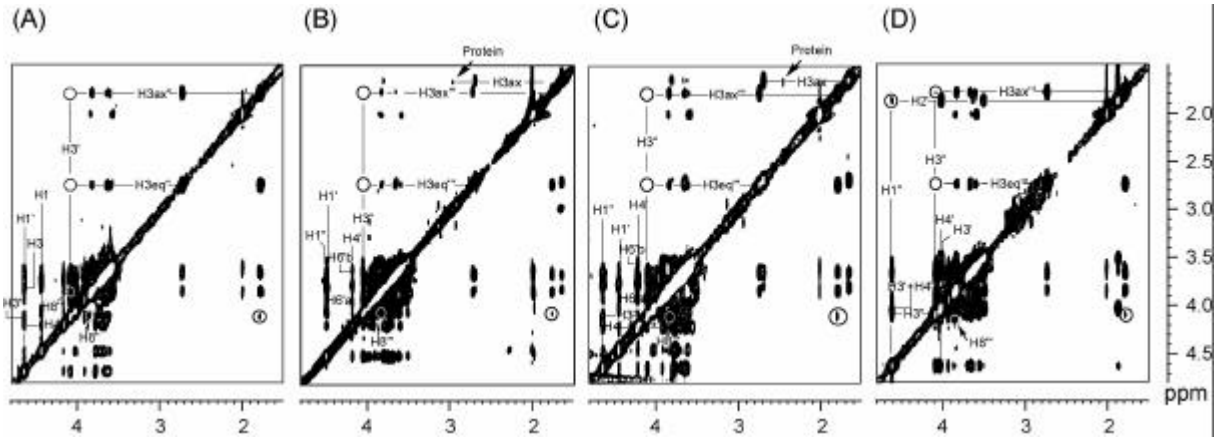


Figure S2. The trNOESY spectra of (A) trisaccharide **5** in the presence of MAG (500 MHz, 288 K), (B) tetrasaccharide **2** (700 MHz, 288 K), (C) tetrasaccharide **3** (500 MHz, 280 K), and (D) pseudo-tetrasaccharide **4** (500 MHz, 288 K). The mixing time was 200 ms. The spectral regions are the same as in Figure S1. The trNOE between H3′ and H3ax″ or between H3″ and H3ax‴ of saccharides **2** to **5** is nearly absent or very weak, and the trNOE between H3 of Gal and H3eq of the $\alpha(2\rightarrow3)$-linked Neu5Ac is completely absent. In contrast, the trNOEs between H3′ and H8″ (trisaccharide **5**) or between H3″ and H8‴ (saccharides **2** to **4**) are stronger in NOESY spectra in the presence of MAG. Open circles highlight these cross peaks.

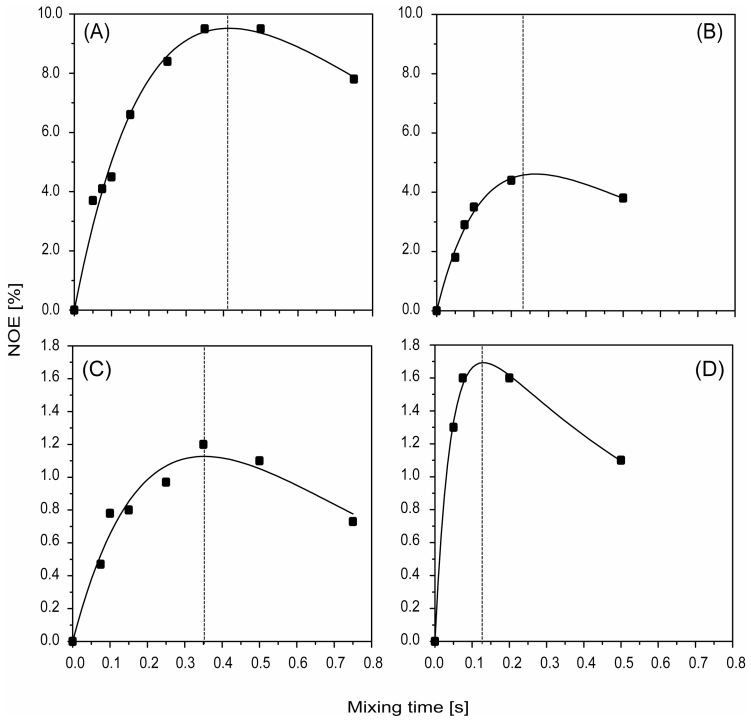
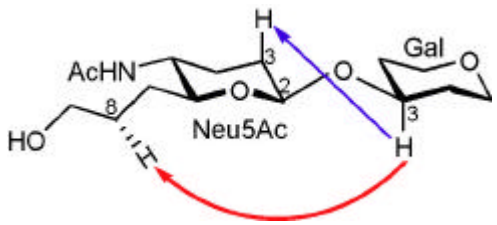


Figure S3. In the upper panel, NOE (left) and trNOE (right) build-up curves of tetrasaccharide **2** for H3″-H3ax‴ in (A) aqueous solution, and (B) in the presence of MAG (288 K, 700 MHz) are shown as a function of the mixing time. It is obvious that the maximum trNOE of H3″-H3ax‴ of tetrasaccharide **2** is lower than the corresponding NOE. This clearly indicates that in the bound state the distance between H3″-H3ax‴ is relatively larger than the H3″-H8‴. The curves have been normalized using the decay of the corresponding diagonal signal as a reference. In the lower panel, the build up curves of the cross peak of H3″-H8‴ of tetrasaccharide **2** in (C) aqueous solution (700 MHz, 288 K), and (D) in the presence of MAG (700 MHz, 288 K) are shown as a function of the mixing time. It is seen that the maximum trNOE of H3″-H8‴ of tetrasaccharide **2** bound to MAG is significantly shifted towards a lower mixing time as compared to the corresponding NOE curve. The maximum intensity of trNOE of H3″-H8‴ is larger than that of the corresponding NOE. The curves have been normalized using the decay of the corresponding diagonal signal as a reference.



Scheme S2.

More importantly, the trNOEs observed across the β(1→3)-glycosidic linkage between the central Gal and Gal*N*Ac for tetrasaccharide **2** (or Gal and Gal for **3**, **4** and **5**) closely match the NOEs observed for the free saccharides **2**, **3**, and **5**. This result indicates that the β(1→3)-glycosidic linkage is less affected upon binding to the protein. Unfortunately, the inter-glycosidic trNOE between H1″- H3′ of tetrasaccharide **2** was difficult to quantify due to severe overlap of resonances from H1″ of Gal and GalNAc anomeric protons (H1′). For the closely related tetrasaccharide **3**, no similar overlap was observed, and hence this molecule was utilized for a detailed analysis of trNOEs. The two trNOEs: H1″- H3′ for tetrasaccharide **3** or H1′-H3 for trisaccharide **5** and H1″- H4′ for tetrasaccharide **3** or H1′-H4 for trisaccharide **5** reflected the orientation of this linkage (Figure S2, Table S2).

In the pseudo-tetrasaccharide **4**, the dideoxy-Gal (Cyc) mimics the Gal*N*Ac residue of tetrasaccharide **2**. The trNOESY spectrum (Figure S2) of the pseudo-tetrasaccharide **4** showed the following inter-glycosidic trNOEs: H1″-H3′, H1″-H4′ and H1″-H2′. Interestingly, the inter-glycosidic trNOE between H1″ and H2′ had gained significant intensity (Figure S2). It was noticed that the H3′ and H4′ of Cyc were nearly coinciding and H2ax′ and H2eq′ of Cyc displayed identical chemical shifts, and hence they together formed a strongly coupled spin system. This makes it difficult to distinguish the corresponding NOEs, and impedes a detailed conformational analysis of this linkage.

The assignment of inter-glycosidic trNOEs across the Neu5Acα(2→6)Gal linkage of both tetrasaccharides **2** and **3** was difficult due to severe signal overlap. Therefore, in addition to the 2D trNOESY experiments we also performed 1D trNOESY experiments. From such an experiment, inter-glycosidic trNOEs between H3ax of α(2→6)-linked Neu5Ac and the protons H6proR′ and H6proS′ of the adjacent Gal*N*Ac residue of **2** were clearly identified. Since a stereospecific assignment of the H6 protons is not available we denote them as H6′a and H6′b.

Table S2. Inter-glycosidic trNOEs of the saccharides **2-5** in 10 mM phosphate buffer, (with 150 mM NaCl, pH* 7.4). Relative NOE intensities are defined as: strong (s) (< 2.5 Å), medium (m) (2.5 to 3.6 Å) and weak (w) (> 3.6 Å). (Lac: Lactic acid residue).

Saccharides	Inter-glycosidic NOEs		Relative intensity
5 (500 MHz, 288 K)	H3′	H3ax″	w
		H3eq″	n.o.
		H8″	s
	H1′	H3	s
		H4	m-w
2 and **3** (**2**: 700 MHz, 288 K and **3**: 500 MHz, 280 K)	H3″	H3ax‴	m
		H3eq‴	w
		H8‴	s
	H1″	H3′	s
		H4′	m-w
	H6′	H3ax	w
		H4′	s-m
	H4′	H6″	m
4 (500 MHz, 288 K)	H3″	H3ax‴	w
		H3eq‴	n.o.
		H8‴	s
	H1″	(H3′+H4′)	s
		(H2ax′+H2eq′)	m
	H6′	-CH^{Lac}	m

Pseudo-tetrasaccharide **4** that carries an (*S*)-lactic acid (Lac) in place of an α(2→6)-linked Neu5Ac residue, showed two inter-glycosidic trNOEs, namely a strong trNOE H6′-CH^{Lac} and a very weak H6″-CH^{Lac} (Table S2). The trNOEs of trisaccharide **6** were of lower absolute intensity than the corresponding oligosaccharides **2** to **5** bound to MAG. So, the contributions from trNOEs and NOEs could not be made explicitly. Therefore, no attempts were made to further analyze the bound conformation of trisaccharide **6**. Since it is well documented that spin diffusion can lead to false distance constraints when determining the bioactive conformation of a ligand, trROESY experiments were performed. The prominent trNOE between H3 of Gal and H8 of Neu5Ac of saccharides **2** to **5** was present in all trROESY spectra, verifying that it was not generated by spin diffusion via protein protons. The trNOE between H1 and H4 of trisaccharide **5** and H1″-H4″ of **3** and **4**, was absent in trROESY spectra, and, therefore, it could be due to spin diffusion. The negative cross peaks H6″-CH^{Lac} and H6′-$CH_3{}^{Lac}$ were also observed in the trROESY spectrum of pseudo-tetrasaccharide **4** in the presence of MAG, indicating that these trNOEs were contaminated via spin diffusion from protein protons.

A full relaxation matrix analysis is performed based on docking models

Prior to docking experiments and full relaxation matrix analysis, a model of MAG was required. Therefore, a homology model was constructed based on the crystal structure of the N-terminal V-set domain of sialoadhesin,[S1] which was co-crystallized with sialyllactose at 1.85 Å resolution (Brookhaven protein data bank acquisition code 1QFO). MAG (Swiss-Prot ID P20917) shares 28% sequence identity with sialoadhesin. The crystal structure has three asymmetric subunits A, B and C. A pair wise sequence alignment was then performed using this 139 amino acid residues containing subunit B as a template (Figure S4) due to its high electron density and minimal temperature factors (B factor). The overall geometric quality of the model was assessed by the program PROCHECK.[S2] In the homology model, 71.1% amino acid residues were found in the most favored regions of the Ramachandran plot, 2.1% were in generously allowed regions and the rest 26.8% were in additional allowed regions. No residues were found in the disallowed regions of the Ramachandran plot. The accuracy of the model was measured by calculating the root mean square (RMS) deviation of the Cα atoms of MAG with respect to their position in the structure of sialoadhesin (1QFO.pdb). The structure consists of a β-sandwich made up by two β-sheets termed ABED and A′GFCC′ (Figure S4). As observed for sialoadhesin,[S1] the G strand of MAG is split into two shorter strands by a short insert. The distinctive "kink" in the G strand occurs at residues 128-129, and the β-sheet is stabilized by hydrogen bonds between Thr 128 and Asn 99, and hydrophobic interactions between Thr 128 and Tyr 116. MAG contains an intrasheet disulfide bond between Cys 42 and Cys 100, located on the B and E strands of the ABED sheet. A comparison of siglec family members has highlighted that amino acid residues involved for Neu5Ac binding are highly conserved. The most crucial residue for the binding of Neu5Ac by MAG is Arg 118, located in the F strand. The missing loops between B and C as well as between F and G were reconstructed using the program COMPOSER (TRIPOS, USA).

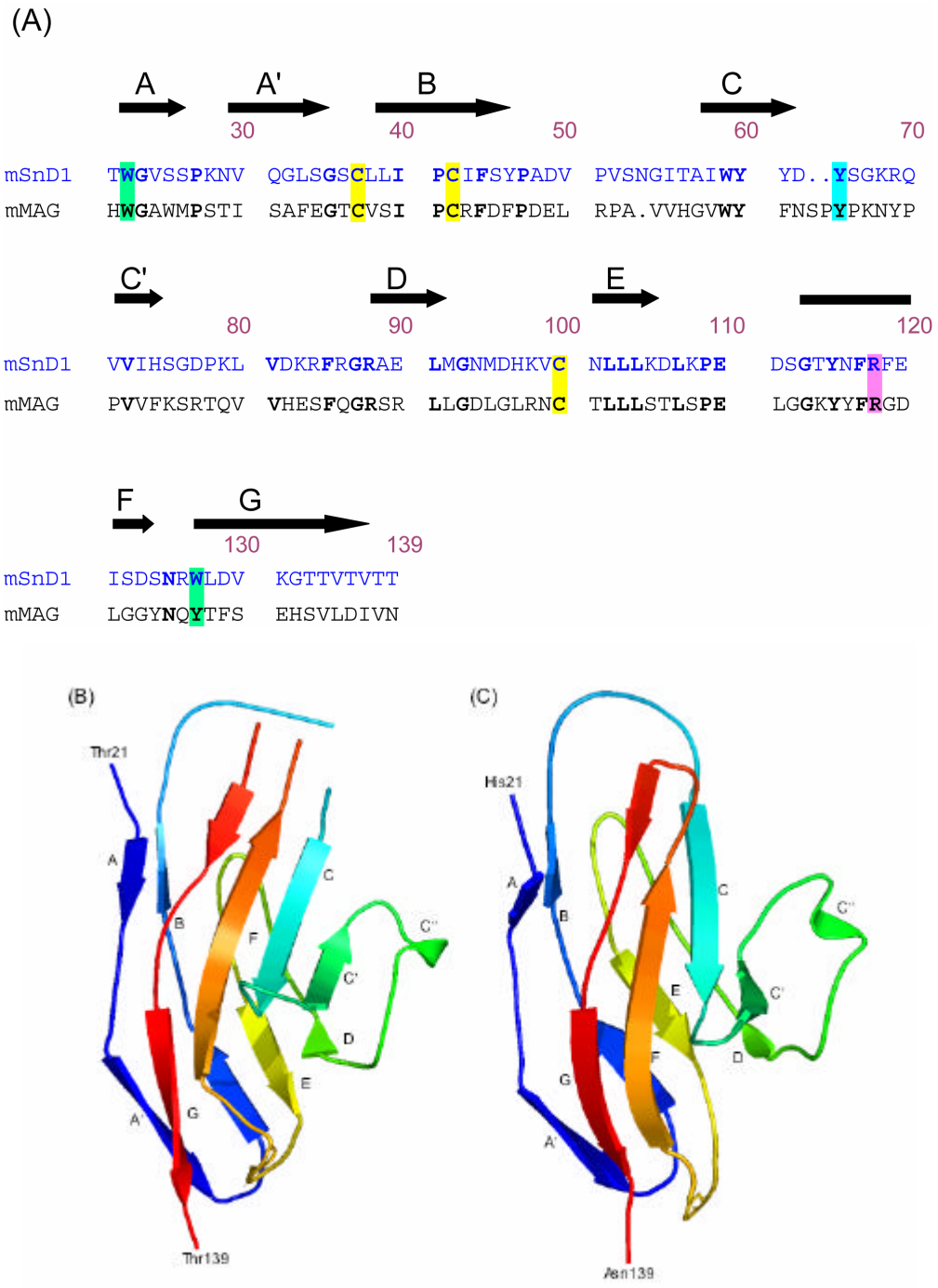


Figure S4. (A) Amino acid sequence alignment of the N-terminal V-set domains of sialoadhesin (1QFO.pdb) and MAG (Swiss-Prot ID P20917). The aromatic residue putatively important in binding the ligands is shown in cyan box, while the siglec hallmark arginine and tryptophans are highlighted here in red and green color box respectively. The cysteines are shown in yellow color. The secondary structure elements are shown as arrows above the sequence. The sequences shown are of: mSnD1, mouse sialoadhesin and mMAG, mouse myelin-associated glycoprotein. In the lower panel, the 3D structure of the N-terminal V-set domain of (B) sialoadhesin and (C) MAG. MAG was modeled using the functionally related

siglec sialoadhesin as a template employing the program COMPOSER as provided by the Sybyl program package. The length of the two proteins is 118 residues each. MAG shares 28% sequence identity with sialoadhesin. Each strand is labeled. The RMS deviation of the Cα atoms of MAG was 1.7 Å with respect to those of the known crystal structure of sialoadhesin.

Employing this homology model of MAG, we performed docking studies with the program AutoDock 3.0.[S3] For the docking, ligands were assumed to be rigid using qualitative inter-glycosidic trNOEs. Thorough conformational analysis of α(2→6)-linkage shows that the ψ value predominantly adopts values around 180°.[S4] Manual docking of tetrasaccharide **3** with Sybyl (TRIPOS, USA) showed that the ψ dihedral angle at the α(2→6)-glycosidic linkage cannot attain a value of 180° because the "trans" orientation at this linkage positions the side chain of Neu5Ac towards the solution. On the other hand, the "gauche" orientation for ψ moves the side chain of α(2→6)-linked Neu5Ac close to the protein surface without affecting the α(2→3)-linked Neu5Ac in its binding pocket. Therefore, a model was constructed for tetrasaccharide **3** with the ϕ, ψ and ω values of ϕ3 = -159°, ψ3 = 81° and ω = 60° at the α(2→6)-linkage and this conformation was then utilized for the AutoDock runs. In all cases AutoDock placed the ligands in the binding pocket in an orientation that is similar to the orientation of the α(2→3)-sialyllactose ligand co-crystallized with sialoadhesin.[S1] The complexes obtained from AutoDock were further energy minimized with Sybyl yielding the bioactive conformations summarized in Table S3. It is predicted that the carboxyl group of the α(2→3)-linked Neu5Ac residue forms a salt bridge with Arg 118, and that the carboxyl group of the α(2→6)-linked Neu5Ac is in contact with the amino group of Lys 67.

Table S3. Dihedral angles (in degrees) at the glycosidic linkages for the bound conformation of saccharides **2** to **5** in the presence of MAG. The dihedral angles (ϕ, ψ, ω) at the glycosidic linkages were defined as: ϕ1 = C1‴-C2‴-O3″-C3″ (Neu5Ac-Gal); ψ1 = C2‴-O2‴-C3″-H3″ (Neu5Ac-Gal); ϕ2 = H1″-C1″-O3′-C3′ (Gal-Gal); ψ2 = C1″-O1″-C3′-H3′ (Gal-Gal); ϕ3 = C1-C2-O6′-C6′ (Neu5Ac-Gal); ψ3 = C2-O2-C6′-C5′ (Neu5Ac-Gal) and ω= O5′-C5′-O6′-C6′.

Ligand	f **1**/y **1**	f **2**/y **2**	f **3**/y **3**	w
2, **3**	-58/-25	68/-40	-159/87	81
4	-59/-26	63/-36		67
5	-64/-17	60/-46		

In order to quantitatively assess the bioactive conformations (Table S3), we performed a full relaxation matrix analysis of the trNOE build-up curves that had been obtained for the saccharides **3**, **4** and **5** employing the program CORCEMA.[S5,S6] The program applies the full relaxation matrix approach and explicitly takes into account protons in the binding site of the protein, as well as effects of chemical and conformational exchange. Experimental and calculated trNOEs build-up curves are compared in Figure S5. Apart from the bioactive conformation of the ligand and its orientation in the binding pocket, the magnitude of the trNOEs depends on a number of parameters such as the dissociation constant K_D, the off-rate constant k_{off}, and the molecular correlation times of the ligand (τ_c^{ligand}) and of the protein ($\tau_c^{protein}$). For the calculation of theoretical trNOEs, overall isotropic motion of the complex with a correlation time of $\tau_c^{protein}$ = 40 ns was assumed. In general, free ligands, such as oligosaccharides, have correlation times in the range of $\tau_c^{ligand} = 10^{-9} - 10^{-10}$ s, whereas bound ligands adapt the correlation time of the receptor protein. The values used for the free and bound correlation times are summarized in Table S4.

For the free trisaccharide ligand **5**, a correlation time of 0.3 ns was estimated from the NOEs observed in the free state (Table S4). Table S5 summarizes inter-proton distances that correspond to experimental trNOEs. Good correspondence between experimental and calculated inter-glycosidic trNOEs (Table S5) shows that the bound conformation of the saccharides **2** to **5** are very close to the conformation that was used in the present calculation (Figure S6).

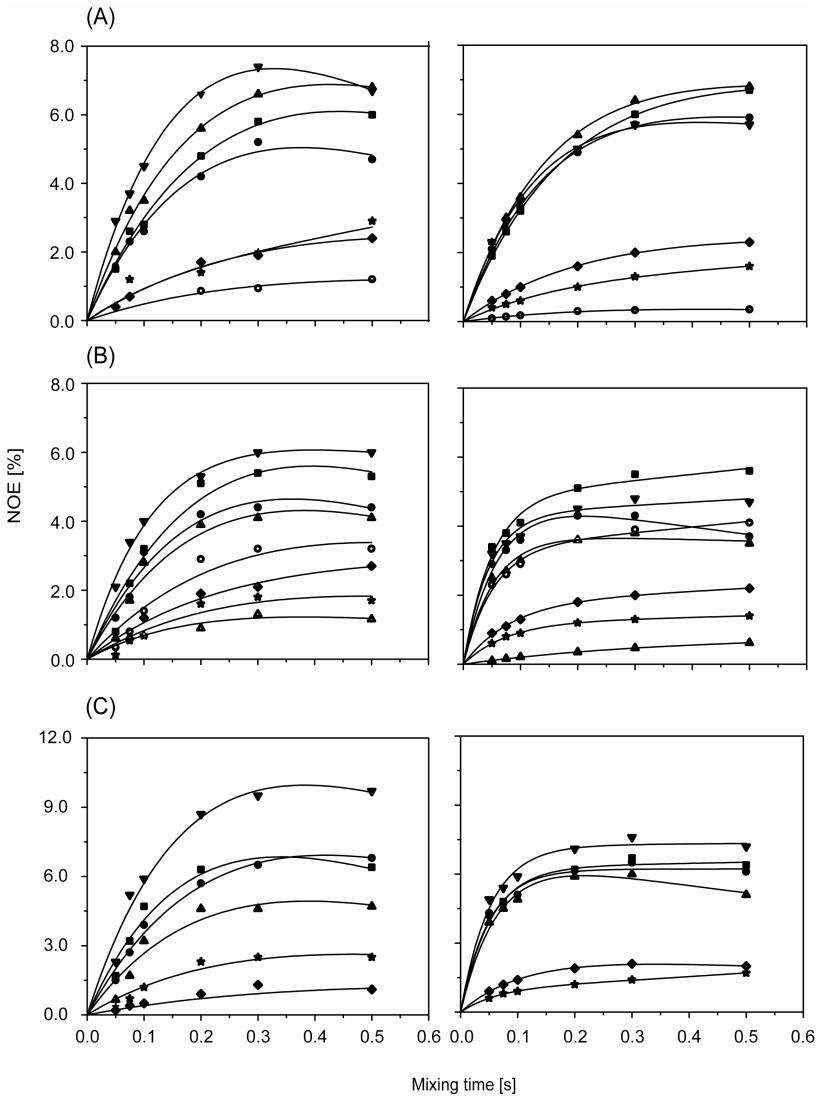


Figure S5. Experimental (left) and theoretical (right) trNOEs (%) build-up curves of (A) trisaccharide **5**, (B) tetrasaccharide **3** and (C) pseudo-tetrasaccharide **4** in the presence of MAG at different mixing times (s). The molar ratio was 1:18, 1:20 and 1:22 for MAG:**5**, MAG:**3** and MAG:**4**, respectively. The initial parameters (K_D, k_{off}, τ_c^{ligand} and $\tau_c^{protein}$) are reported in Table S4. The curves represent following trNOEs: (A) H1-H3 (■), H1′-H3′ (●), H3′-H4′ (▲), H1′-H3 (▼), H1′-H4 ( ), H3′-H8″ ( ) and H3′-H3ax″ (○); (B) H1″-H3″ (■), H4′-H6′b (●), H4′-H6′a (▲), H1″-H3′ (▼), H1″-H4′ ( ), H4′-H6″ ( ) H3″-H8‴ (○) and H3″-H3ax‴ (△); (C) H1″-H3″ (■), H1″-H5″ (●), H6′-CH^{Lac} (▲), H1″-(H3′+H4′) (▼), H1″-(H2ax′+H2eq′) ( ) and H3″-H8‴ ( ).

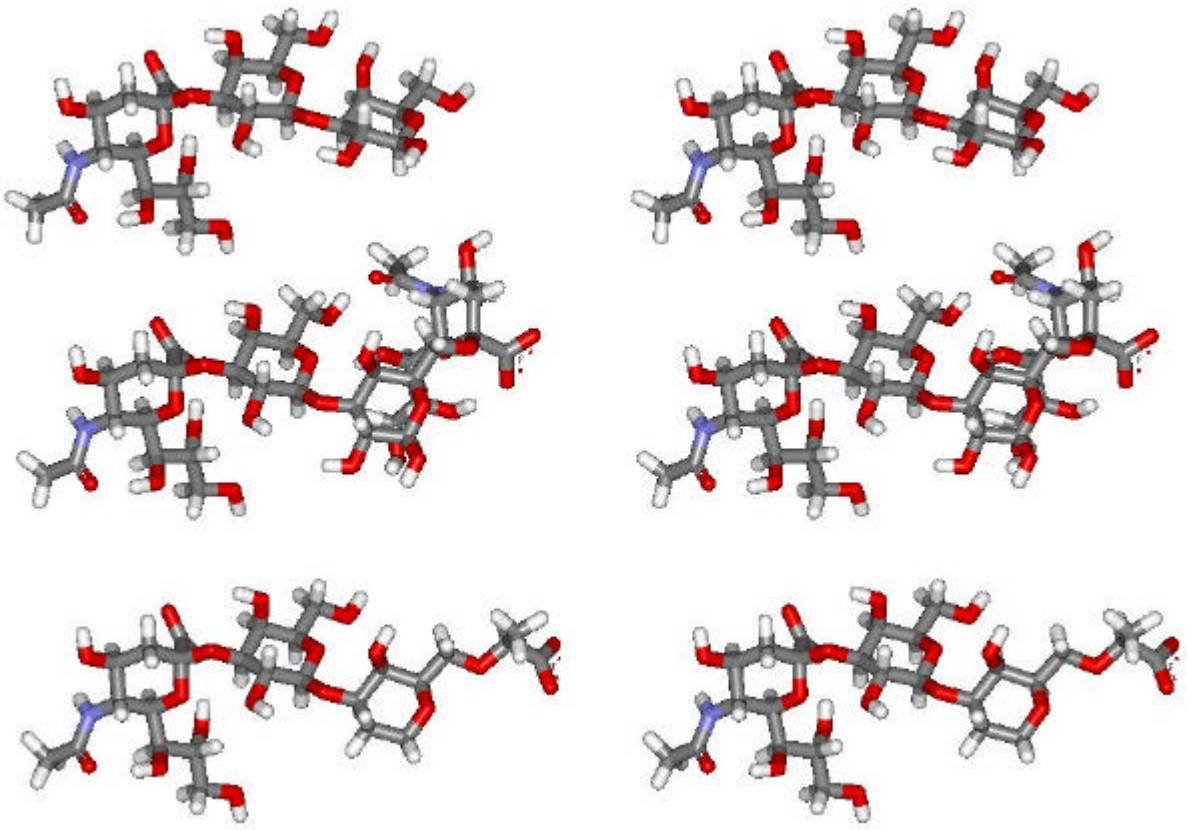


Figure S6. Bioactive conformation of (top) trisaccharide **5**, (middle) tetrasaccharide **3** and (bottom) pseudo-tetrasaccharide **4**. In the bioactive conformation, the Neu5Acα(2→3)Gal orientation is very similar to the one found in sLe^x bound to E-selectin. The orientation of the α(2→6)-linkage of tetrasaccharide **3** is set to "gt" conformation (ω = O5′-C5′-O6′-C6′).[S4]

Table S4. The K_D, k_{off}, τ_c^{ligand} and $\tau_c^{protein}$ values of the saccharides **3**, **4** and **5** were used for the theoretical trNOE (CORCEMA) calculations.

Ligand	**K_D (mM)**	**k_{off} (s^{-1})**	**k_{on} ($M^{-1}s^{-1}$)**	**t_c^{ligand} (ns)**	**$t_c^{protein}$ (ns)**
Trisaccharide **5**	390	40	10^5	0.3	40
Tetrasaccharide **3**	180	10	$5{\cdot}10^4$	0.5	40
Pseudo-tetrasaccharide **4**	180	12	$6{\cdot}10^4$	0.35	40

Table S5. Interproton distances calculated for the bioactive conformations (Figure S6) of the saccharide ligands (A) trisaccharide **5**, (B) tetrasaccharide **3** and (C) pseudo-tetrasaccharide **4** in the presence of MAG. Interproton distances for the proton pairs were measured from the calculated trNOEs, and the R-factors indicate the deviation between experimental and calculated trNOEs.

(A)

Restraints	Distance (Å)	R-factor
H1-H3	2.6	0.10
H1′-H3′	2.5	0.20
H3′-H4′	2.5	0.04
H1′-H3	2.4	0.21
H1′-H4	3.4	0.08
H3′-H3ax″	4.2	0.67
H3′-H8″	2.6	0.42

(B)

Restraints	Distance (Å)	R-factor
H1″-H3″	2.6	0.39
H1″-H3′	2.5	0.18
H1″-H4′	3.7	0.20
H4′-H6′b	2.5	0.36
H4′-H6′a	3.5	0.32
H4′-H6″	3.3	0.28
H3″-H3ax‴	4.3	0.57
H3″-H8‴	2.7	0.71

(C)

Restraints	Distance (Å)	R-factor
H1″-H3″	2.5	0.24
H1″-H5″	2.4	0.30
H6′b-CH^{Lac}	2.4	0.56
H1″-(H3′+H4′)	H1″-H3′: 2.4 H1″-H4′: 3.6	0.24
H1″-H2′	H1″-H2ax′: 4.4 H1″-H2eq′: 3.7	1.00
H3″-H8‴	2.8	0.40

Implications of binding affinities

Both tetrasaccharides **2** and **3** showed higher affinities to MAG in comparison to the trisaccharide **5** (rIP = 1.0) (Table S6). The AutoDock model revealed that the 2nd carboxylate group of Neu5Ac at the α(2→6)-linkage forms a salt bridge with the NH_3^+ group of Lys 67. To verify the prediction of the AutoDock model, two strategies were used to evaluate the

contribution of this additional Neu5Ac for binding to MAG. Firstly, the experiments were performed with K67A mutant MAG and tetrasaccharide **2**, which reduced the binding affinity of tetrasaccharide **2** ca. 50% (rIP = 1.4) (Table S6). Secondly, the replacement of the α(2→6)-linked Neu5Ac of tetrasaccharide **2** or **3** with (*S*)-Lac in pseudo-tetrasaccharide **4** indicated similar affinity to MAG (rIP = 4.1). Therefore, it is possible that the interaction between this additional carboxylate group at the α(2→6)-linked moiety and MAG is important for binding. Interestingly, in the absence of α(2→3)-linked Neu5Ac in trisaccharide **6**, the binding affinity to MAG was significantly weaker (rIP = 0.2) (Table S6). This result is in accordance with the study of the binding affinity (IC_{50}) of these oligosaccharides with MAG by Schnaar and co-workers,[S7] which also show that the trisaccharide **6** is ca. 33 times weaker binding to MAG in comparison to tetrasaccharide **2**. This result concludes that the salt bridge between the carboxyl group of α(2→3)-linked Neu5Ac and Arg 118 of MAG is a prerequisite for binding to MAG.[S8]

Table S6. Relative inhibitory potencies (rIPs) of saccharides **2** to **6**. The rIPs of each inhibitor are calculated by dividing the IC_{50} of the reference compound trisaccharide **5** by the IC_{50} of the compound of interest. The rIPs of the saccharides **2** to **4** that inhibit better than the reference compound **5** are greater than 1.0 while the rIP for saccharide **6**, which is a weaker inhibitor is less than 1.0. Mutation of Lys 67 (K67A) (unpublished data) shows the significant reduction in binding. It is suggested that this loss of activity is due to the loss of electrostatic interaction between Lys 67 and the carboxylate group of α(2→6)-linked Neu5Ac/Lac. (WT: Wild type).

Ligand	**WT MAG**	**K67A MAG**
Trisaccharide **5**	1.0	1.0
Tetrasaccharide **2**	3.3	1.4
Tetrasaccharide **3**	4.2	not measured
Pseudo-tetrasaccharide **4**	4.1	not measured
Trisaccharide **6**	0.2	not measured

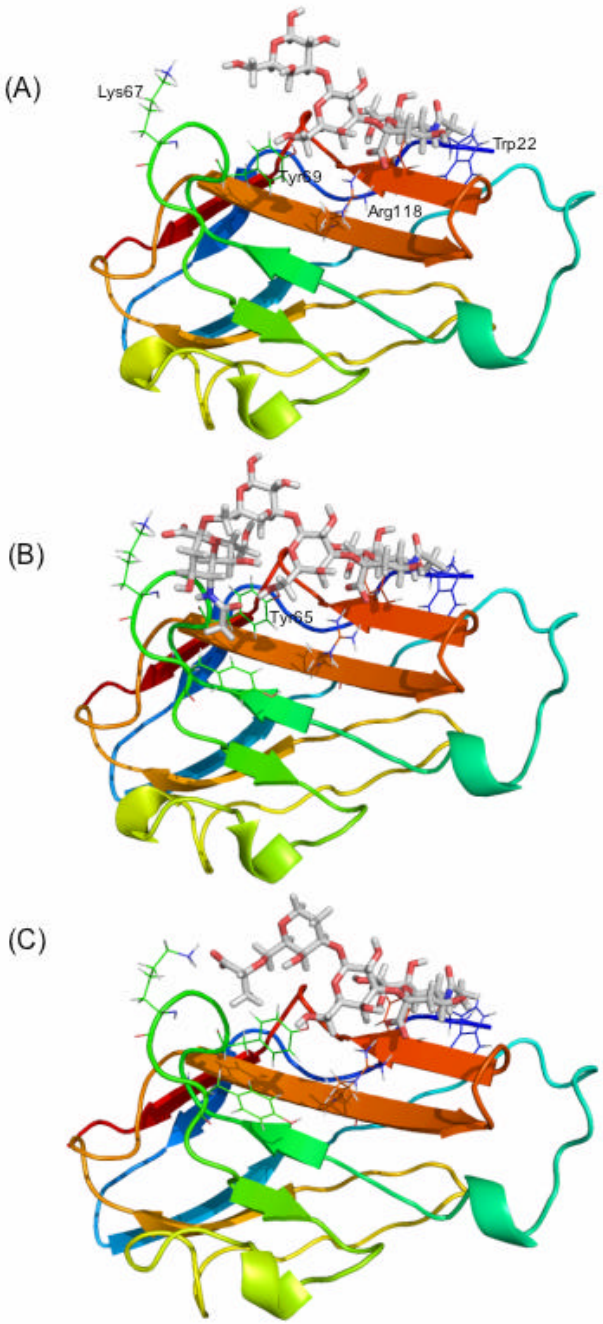


Figure S7. The structure of (A) trisaccharide **5**, (B) tetrasaccharide **3** and (C) pseudo-tetrasaccharide **4** in the binding site of MAG. The corresponding dihedral angles at the glycosidic linkages of the saccharides are reported for the theoretical trNOE calculations using CORCEMA program (Table S4). The α(2→3)-glycosidic linkage prefers "syn" conformation. The dotted line indicates the strong electrostatic interaction between MAG amino acids and saccharides. The strong electrostatic interaction between the NH_3^+ group of Lys 67 and the carboxyl group of α(2→6)-linked Neu5Ac/(S)-Lac increases the binding affinity of tetrasaccharide **3** or pseudo-tetrasaccharide **4** to MAG. The graphics were produced with MacPyMOL, DeLano Scientific, 2006.

Table S7. Bioactive conformations of α(2→3)-linked Neu5Ac residues (Sia) of oligosaccharide ligands bound to receptor proteins.

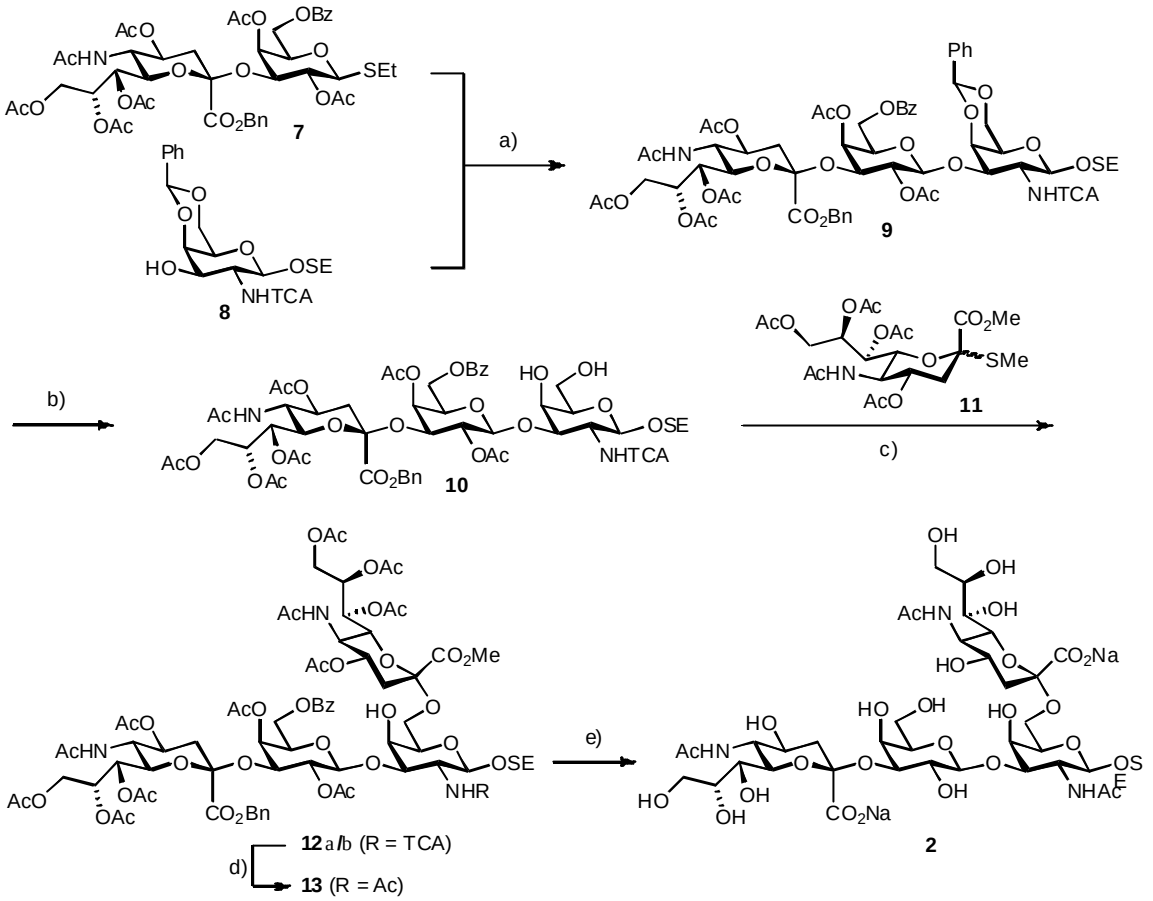
Protein	Ligand	ϕ [°] *	ψ [°]	$H3^{Gal}$ - $H3^{Sia}{}_{ax}$ [Å]	$H3^{Gal}$ - $H8^{Sia}$ [Å]	Reference	PDB Access Code / Resolution [Å]
Maackia amurensis lectin	sialyllactose	-70.3 -70.5	+2.9 +2.7	4.18 4.18	3.85 3.84	S9	1DBN / 2.75
Clostridum Botulinum Neurotoxin B	sialyllactose	+19.7	+33.7	2.55	4.11	S10	1F31 / 2.60
Tetanus Toxin Hc Fragment	GT1b	-71.6[a] -32.0[b]	-37.2[a] -19.9[b]	3.15[a] 4.39[b]	6.43[a] 4.56[b]	S11	1FV2 / 2.5
Tetanus Toxin Hc Fragment	GT1b	-42.6[a] -67.7[a] -27.0[b] -34.5[b]	-72.4[a] -24.7[a] -16.0[b] -26.2[b]	3.75[a] 3.31[a] 4.19[b] 4.22[b]	5.48[a] 5.53[a] 4.39[b] 4.39[b]	S11	1FV3 / 2.3
P-selectin	sialyl $Lewis^x$	-55.0 -65.4 -65.2	-12.9 -8.4 -15.8	4.27 4.22 4.21	3.55 3.54 2.84	S12	1G1R / 3.4
P-selectin	PSGL-1	-61.1 -59.6	-0.9 +2.6	4.30 4.29	3.11 2.94	S12	1G1S / 1.9
E-selectin	sialyl $Lewis^x$	-64.7	-11.6	4.18	3.00	S12	1G1T / 1.5
Swine Hemagglutinin H9	LSTa	-70.6	-22.7	4.13	2.91	S13	1JSH / 2.4
Avian Hemagglutinin H5	LSTa	-174.0	-23.2	2.10	4.17	S13	1JSN / 2.4
Sialoadhesin	sialyllactose	-69.9 -69.5	-17.3 -20.9	4.12 4.11	2.82 2.76	S1	1QFO / 1.85
Polyomavirus	sialyllactose	-39.0 -61.2 -57.5	2.2 13.5 16.6	4.26 4.15 4.21	3.60 5.10 3.97	S14	1SID / 3.65
Polyomavirus	disialylated oligosaccharide	-48.0 -49.9 -45.2	2.5 5.4 8.8	4.25 4.22 4.27	4.09 3.52 3.56	S14	1SIE / 3.65
Mutant Mannose Binding Protein	sialyl $Lewis^x$	-67.2 -68.5 -51.4	-18.2 -26.1 -38.6	4.16 4.18 4.31	3.30 2.91 3.47	S15	2KMB / 2.00
Cholera Toxin B-Pentamer	GM1 pentasaccharide	-171.0 -171.8 -170.0 -175.6 -171.8	-28.4 -15.7 -33.5 -26.7 -26.9	2.31 2.39 2.23 2.32 2.22	4.48 4.29 4.47 4.63 4.51	S16	2CHB / 2.00
Cholera Toxin B-Pentamer	GM1 pentasaccharide	-171.4 -168.4 -172.2 179.2 -176.9	-23.3 -15.5 -20.6 -15.5 -15.8	2.41 2.27 2.09 2.19 2.13	4.50 4.01 4.29 4.38 4.40	S17	3CHB / 1.25
E-selectin	sialyl $Lewis^x$	-76	6	4.28	2.72	S18	NMR
E-selectin	sialyl $Lewis^x$	-58	-22	4.36	2.89	S19	NMR
E-selectin	sialyl $Lewis^x$	-43	-12	4.48	3.47	S20	NMR
Aleuria aurantia agglutinin	sialyl $Lewis^x$	-62	-4	4.32	2.82	S21	NMR
MAG	**2**, **3**, **4**, **5**	cf. Table S3				this study	NMR

* The dihedral angles ϕ and ψ are defined as follows: $\phi = C1^{Sia}\text{-}C2^{Sia}\text{-}O2^{Sia}\text{-}C3^{Gal}$, $\psi = C2^{Sia}\text{-}O2^{Sia}\text{-}C3^{Gal}\text{-}H3^{Gal}$. [a] orientation of the Neu5Ac residue in the Neu5Acα(2→8)Neu5Acα(2→3) fragment; [b] orientation of the terminal Neu5Acα(2→3) residue.

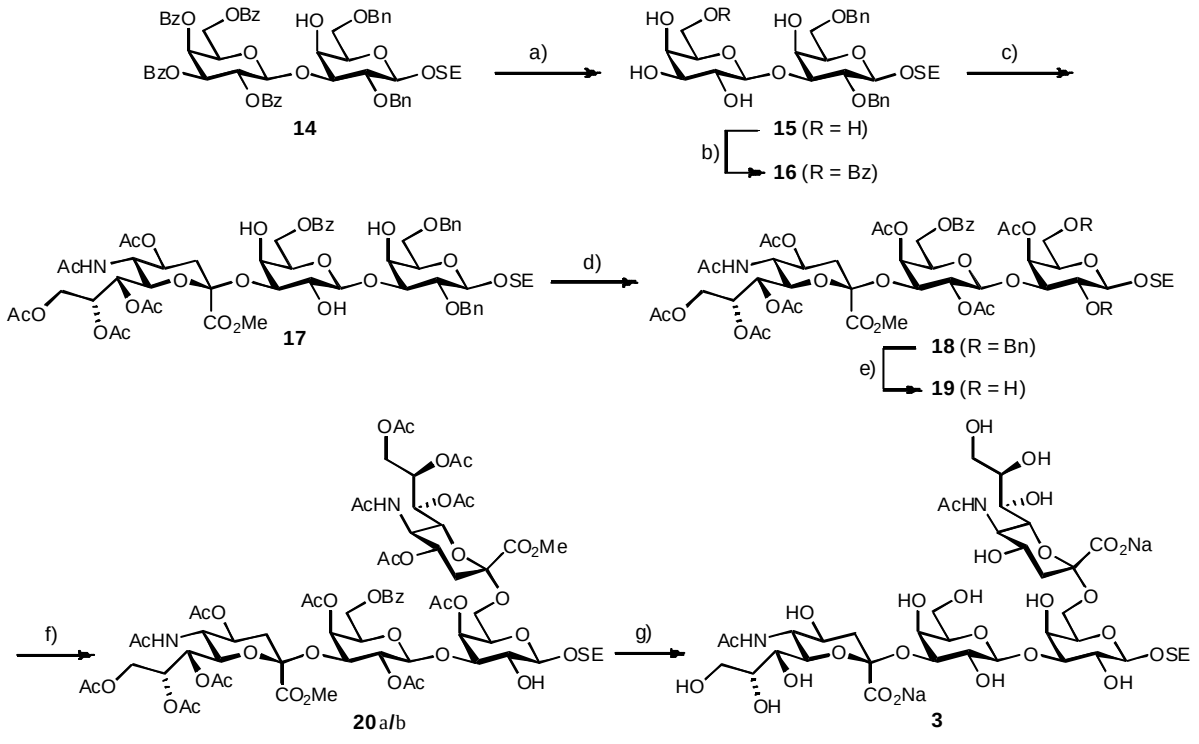
Experimental Procedures

Synthesis of GQ1ba fragments 2 and 3 (*Schemes S3 & S4*) and the tetrasaccharide mimetic 4 (*Scheme S5*)

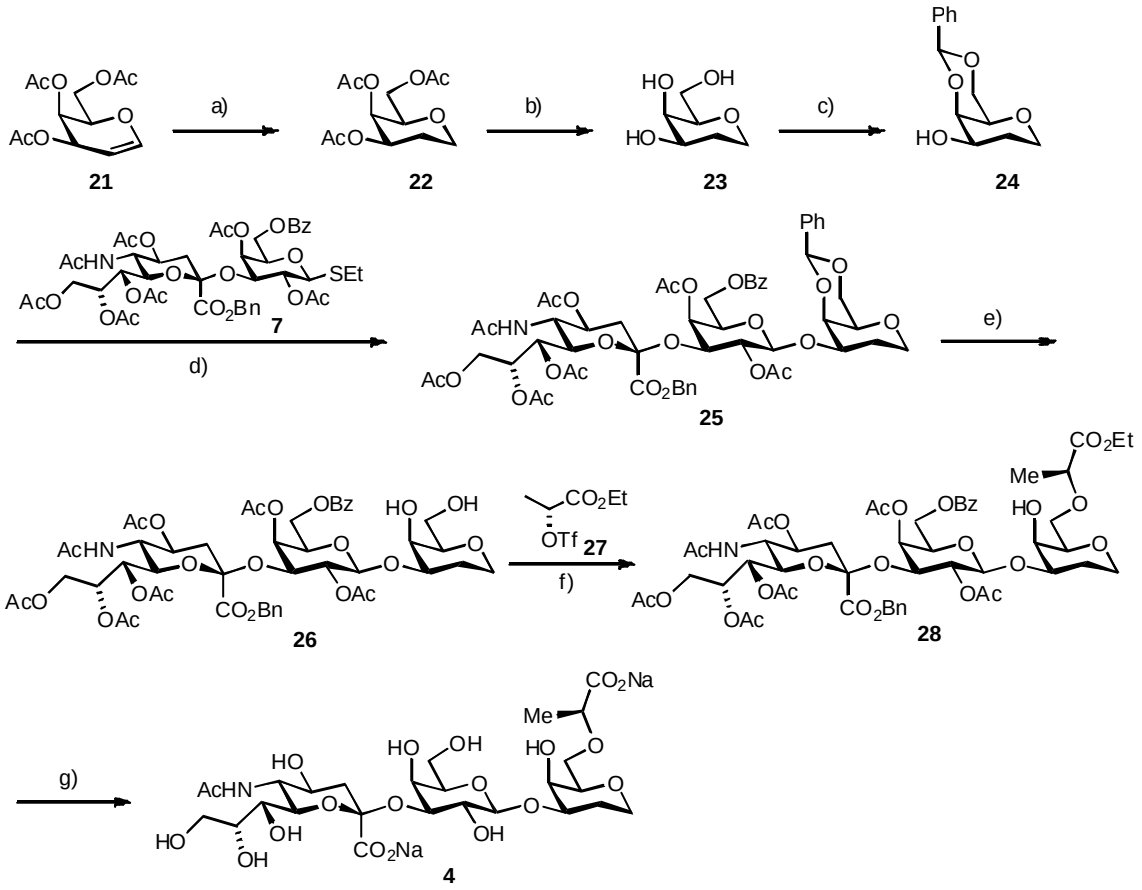
General Methods. NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^1H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HMQC, TOCSY). Chemical shifts are expressed in ppm using residual $CHCl_3$, CHD_2OD and HDO as references. Optical rotations were measured using a Perkin-Elmer Polarimeter 241. Low resolution LC/MS analysis were carried out using a Waters Alliance 2690 LC system equipped with a photodiode array detector and a Micromass Quattro II mass spectrometer. The spectra were recorded in positive EI mode. The LC method consisted of a separation column (YMC ODS AQ 12.5 cm length, 2 mm i.d.) held at 40°C. The mobile phase was MeCN/H_2O with the addition of 0.05% trifluoroacetic acid using a flow rate of 0.5 mL/min. The linear gradient run from 0% to 100% MeCN in 15 min followed by 5 min at 100% MeCN before returning to initial conditions. LC/HRMS analysis were carried out using a Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. All spectra were recorded in positive EI mode. The LC method consisted of a separation column (YMC ODS AQ 12.5 cm length, 2 mm i.d.) held at room temperature The mobile phase was MeCN/H_2O with the addition of 0.5% formic acid using a flow rate of 0.2 mL/min. The linear gradient run from 5% to 95% MeCN in 10 min followed by 8 min at 95% MeCN before returning to initial conditions. Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel (Uetikon, 40-60 mesh). Methanol was dried by refluxing with sodium methoxide and distilled immediately before use. Pyridine was freshly distilled under argon over KOH. Dichloromethane (DCM), dichloroethane, acetonitrile, toluene, and benzene were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). Molecular sieves (3 Å) were activated in vacuo at 500°C for 2 h immediately before use.



Scheme S3. a) DMTST, MS 3Å, DCM, 0°C, 2 d, 46%; b) 80% aq. AcOH, 60°C, 3 h, 84%; c) NIS, TfOH, MS 3Å, MeCN, -30°C, 21 h, **12**a : 52%, **12**b : 36%; d) Bu_3SnH, AIBN, benzene, 80°C, 3 h, 73%; (e) NaOMe, MeOH, r.t., 1 d, then aq. NaOH, r.t., 1 d, 76%.



Scheme S4. a) NaOMe, MeOH, r.t., 2 h, 85%; b) BzCN, NEt_3, MeCN, -40°C, 2 h, 66%; c) **11**, NIS, TfOH, MS 3Å, MeCN, -30°C, 16 h, 54%; d) Ac_2O, pyridine, r.t., 16 h, 80%; e) H_2, 10% Pd-C, EtOH/AcOH, 50°C, 4 h, 76%; f) **11**, NIS, TfOH, MS 3Å, MeCN, -40°C, 16 h, **20**a : 38%, **20**b : 12%; g) i. NaOMe, MeOH, r.t., 1 d, ii. aq. LiOH, r.t., 1 d, iii. Dowex 50x8 (Na^+), 46%.



Scheme S5. a) H_2 (1 bar), 10% Pd-C, MeOH, 3 h, 97%; b) NaOMe, MeOH, r.t., 3 h, 99%; c) $PhCH(OMe)_2$, cat. *p*-TsOH, MeCN, r.t., 18 h, 84%; d) DMTST, MS 3Å, DCM, 0°C, 2 d, 79%; e) 80% aq. AcOH, 60°C, 3 h, 89%; f) i. Bu_2SnO, benzene, 80°C, 3 h, ii. **27**, CsF, DME, r.t., 1 d, 83%; g) NaOMe, MeOH, r.t., 1 d, then aq. NaOH, r.t., 7 h, 81%.

2-(Trimethylsilyl)ethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-a-D-*galacto*-2-nonulopyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-b-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-b-D-galactopyranoside (9). A mixture of donor **7**[S22] (290 mg, 0.300 mmol), acceptor **8**[S23] (308 mg, 0.600 mmol) and activated powdered molecular sieves 3Å (2.0 g) in DCM (20 mL) was stirred for 2 h at r.t. under argon. Then DMTST (352 mg, 1.20 mmol) was added in one portion at 0°C. The reaction mixture was stirred at 0°C for 2 d, diluted with DCM (10 mL) and filtered through a

pad of Celite. The Celite was washed with DCM (3 × 10 mL) and the combined filtrates were washed with saturated aqueous $KHCO_3$ (10 mL) and H_2O (10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (DCM/MeOH 25:1 to 20:1) to afford disaccharide **9** (197 mg, 46%) as a colorless foam.

$[\alpha]_D$ +15.3 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.94 (m, 2H, C*H*$_2$Si), 1.67 (t, *J* = 12.4 Hz, 1H, Sia-H3a), 1.84, 2.00, 2.07, 2.09, 2.11, 2.14, 2.20 (7s, 21H, 7 $COCH_3$), 2.62 (dd, *J* = 4.6, 12.7 Hz, 1H, Sia-H3b), 3.24 (m, 1H, GalN-H5), 3.49 (dd, *J* = 2.4, 10.9 Hz, 1H, Sia-H6), 3.53 (m, 1H, OC*H*$_2$-Ha), 3.77 (m, 1H, GalN-H2), 3.79 (m, 1H, GalN-H6a), 3.99 (m, 1H, OC*H*$_2$-Hb), 4.01 (m, 1H, Sia-H9a), 4.02 (m, 1H, Sia-H5), 4.11 (m, 1H, Gal-H5), 4.19 (m, 1H, GalN-H6b), 4.21 (m, 1H, Gal-H6a), 4.29 (dd, *J* = 2.6, 12.6 Hz, 1H, Sia-H9b), 4.35 (d, *J* = 3.3 Hz, 1H, GalN-H4), 4.53 (dd, *J* = 3.4, 11.2 Hz, 1H, GalN-H3), 4.55 (dd, *J* = 7.3, 11.0 Hz, 1H, Gal-H6b), 4.68 (dd, *J* = 3.4, 9.9 Hz, 1H, Gal-H3), 4.85 (m, 1H, Sia-H4), 4.90 (d, *J* = 7.9 Hz, 1H, Gal-H1), 4.95 (d, *J* = 10.3 Hz, 1H, NH), 5.02 (A of AB, *J* = 11.9 Hz, 1H, PhC*H*$_2$), 5.03 (d, *J* = 8.5 Hz, 1H, GalN-H1), 5.13 (dd, *J* = 8.0, 9.9 Hz, 1H, Gal-H2), 5.17 (d, *J* = 3.3 Hz, 1H, Gal-H4), 5.35 (m, 1H, Sia-H7), 5.38 (B of AB, *J* = 11.9 Hz, 1H, PhC*H*$_2$), 5.41 (ddd, *J* = 2.8, 4.7, 9.1 Hz, 1H, Sia-H8), 5.49 (s, 1H, PhC*H*), 6.95 (d, *J* = 7.0 Hz, 1H, NH), 7.28-7.29, 7.33-7.40, 7.45-7.49, 7.53-7.62, 8.09-8.11 (m, 15H, 3 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.6 ($SiMe_3$), 18.9 (CH_2Si), 20.5, 20.6, 20.6, 20.7, 21.2, 22.9 (7C, 7 CO*C*H_3), 30.3 (Sia-C3), 48.8 (Sia-C5), 54.9 (GalN-C2), 61.7, 62.1 (Gal-C6, Sia-C9), 66.4 (Sia-C7), 66.7 (GalN-C5), 67.1 (OCH_2), 67.3, 67.5 (Gal-C4, Sia-C8), 68.3 (Ph*C*H_2), 68.6 (GalN-C6), 69.0 (Sia-C4), 69.9 (Gal-C2), 70.5 (Gal-C5), 71.5 (Gal-C3), 72.0 (Sia-C6), 74.6 (GalN-C3), 75.3 (GalN-C4), 92.2 (*C*Cl_3), 96.7 (Sia-C2), 98.0 (GalN-C1), 100.5 (Gal-C1), 101.0 (Ph*C*H), 126.1, 127.8, 128.2, 128.3, 128.5, 128.5, 128.6, 128.6, 129.5, 133.3, 134.8, 138.1 (18C, 3 C_6H_5), 161.9, 165.7, 167.0, 170.8, 170.9, 171.7, 172.3, 173.2, 173.5 (10C, 10 CO); HRMS (FAB) Calcd for $C_{63}H_{81}Cl_3N_3O_{26}Si$ $[M+NH_4]^+$: 1428.3943; Found: 1428.3984.

2-(Trimethylsilyl)ethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α**-D-*galacto*-2-nonulopyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-**β**-D-galactopyranosyl)-(1→3)-2-deoxy-2-trichloroacetamido-**β**-D-galactopyranoside (10)**. Compound **9** (190 mg, 0.135 mmol) was dissolved in 80% aqueous acetic acid (5 mL) and stirred for 4 h at 60°C. The solvent was concentrated *in vacuo* and the residue co-evaporated with toluene (2 × 5 mL). The remaining solid was purified by column chromatography on silica gel (DCM/MeOH 20:1 to 10:1) to give trisaccharide **10** (154 mg, 86%) as a colorless solid.

$[\alpha]_D$ +18.1 (*c* 1.02, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ -0.04 (s, 9H, $SiMe_3$), 0.87-0.97 (m, 2H, CH_2Si), 1.65 (t, *J* = 12.4 Hz, 1H, Sia-H3a), 1.83, 1.98, 2.05, 2.06, 2.10, 2.15, 2.20 (7s, 21H, 7 $COCH_3$), 2.61 (dd, *J* = 4.6, 12.7 Hz, 1H, Sia-H3b), 2.98 (s, 1H, OH), 3.48 (dd, *J* = 2.6, 10.8 Hz, 1H, Sia-H6), 3.49 (m, 1H, GalN-H5), 3.52-3.59 (m, 2H, GalN-H6a, OCH_2-Ha), 3.62 (m, 1H, GalN-H2), 3.83 (m, 1H, GalN-H6b), 3.96 (m, 1H, OCH_2-Hb), 3.98-4.04 (m, 2H, Sia-H5, Sia-H9a), 4.09-4.10 (m, 2H, GalN-H4, Gal-H5), 4.30 (dd, *J* = 2.7, 12.6 Hz, 1H, Sia-H9b), 4.33-4.38 (m, 2H, Gal-H6), 4.43 (dd, *J* = 3.2, 10.8 Hz, 1H, GalN-H3), 4.67 (dd, *J* = 3.4, 10.1 Hz, 1H, Gal-H3), 4.84 (d, *J* = 8.0 Hz, 1H, Gal-H1), 4.86 (m, 1H, Sia-H4), 4.92 (d, *J* = 10.3 Hz, 1H, NH), 4.96 (d, *J* = 8.3 Hz, 1H, GalN-H1), 5.03 (A of AB, *J*=12.0 Hz, 1H, $PhCH_2$), 5.08 (dd, *J* = 8.0, 10.1 Hz, 1H, Gal-H2), 5.13 (d, *J* = 3.2 Hz, 1H, Gal-H4), 5.34 (dd, *J* = 2.6, 9.0 Hz, 1H, Sia-H7), 5.38 (B of AB, *J* = 12.0 Hz, 1H, $PhCH_2$), 5.43 (ddd, *J* = 2.8, 5.0, 9.0 Hz, 1H, Sia-H8), 6.89 (d, *J* = 7.1 Hz, 1H, NH), 7.35-7.41, 7.47-7.50, 7.59-7.62, 8.06-8.08 (m, 10H, 2 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.5 ($SiMe_3$), 18.0 (CH_2Si), 20.7, 20.7, 20.8, 20.8, 22.2, 22.4, 23.1 (7 $COCH_3$), 37.5 (Sia-C3), 48.8 (Sia-C5), 55.0 (GalN-C2), 62.2, 62.3 (3C, GalN-C6, Gal-C6, Sia-C9), 66.8 (Sia-C7), 67.1 (OCH_2), 67.4, 67.6 (Gal-C4, Sia-C8), 68.1 (GalN-C4), 68.4 ($PhCH_2$), 69.0 (Gal-C2), 69.6 (Sia-C4), 70.9 (Gal-C5), 71.2 (Gal-C3), 72.1 (Sia-C6), 73.5 (GalN-C5), 77.8 (GalN-C3), 92.0 (CCl_3), 96.8 (Sia-C2), 98.3 (GalN-C1), 100.7 (Gal-C1), 128.4, 128.7, 128.8, 129.2, 130.4, 130.8, 133.1, 134.5 (12C, 2 C_6H_5), 161.8, 165.8, 167.3, 169.0, 169.5, 170.1, 170.3, 170.5, 171.3, 171.6 (10 CO); HRMS (FAB) Calcd for $C_{56}H_{77}Cl_3N_3O_{26}Si$ $[M+NH_4]^+$: 1340.3630; Found: 1340.3635.

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α**-D-*galacto*-2-nonulopyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-**β**-D-galactopyranosyl)-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α- and β-D-*galacto*-2-nonulopyranosynate)]-(2→6)-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (12)**. To a solution of **10** (99.0 mg, 74.7 μmol) and donor **11**[S24] (78.0 mg, 0.150 mmol) in MeCN (3 mL) was added activated powdered molecular sieves 3Å (500 mg). The reaction mixture was stirred at r.t. under argon for 2 h and then cooled to -30°C. After the addition of NIS (67.4 mg, 0.299 mmol) and TfOH (2.6 μL, 29.9 μmol), stirring was continued for 21 h at -30°C. Then the mixture was diluted with DCM (10 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3 × 10 mL), and the combined filtrates were washed with 20% aqueous $Na_2S_2O_3$ (5 mL), saturated aqueous $KHCO_3$ (10 mL), and H_2O (10 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by silica

gel chromatography (DCM/MeOH 20:1 to 5:1) to afford **12**a (70.3 mg, 52%) and **12**b (48.4 mg, 36%) as colorless foams.

12a : $[\alpha]_D$ -2.3 (*c* 0.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ -0.02 (s, 9H, $SiMe_3$), 0.86-0.93 (m, 2H, C*H*$_2$Si), 1.64 (t, *J* = 12.4 Hz, 1H, Sia-H3a), 1.82, 1.87, 1.97, 2.00, 2.02, 2.05, 2.05 (7s, 21H, 7 $COCH_3$), 2.06 (m, 1H, Sia'-H3a), 2.08, 2.09, 2.12, 2.15, 2.20 (5s, 15H, 5 $COCH_3$), 2.55 (dd, *J* = 4.6, 12.8 Hz, 1H, Sia'-H3b), 2.61 (dd, *J* = 4.6, 12.7 Hz, 1H, Sia-H3b), 2.68 (s, 1H, OH), 3.46 (dd, *J* = 2.5, 10.7 Hz, 1H, Sia-H6), 3.55 (m, 1H, OC*H*$_2$-Ha), 3.61 (m, 1H, GalN-H5), 3.69 (m, 1H, GalN-H6a), 3.71 (ddd, *J* = 7.7, 8.1, 10.5 Hz, 1H, GalN-H2), 3.80 (s, 3H, OCH_3), 3.85 (dd, *J* = 5.7, 9.9 Hz, 1H, GalN-H6b), 3.93-4.04 (m, 4H, Sia-H5, Sia-H9a, Sia'-H5, OC*H*$_2$-Hb), 4.06-4.11 (m, 3H, Gal-H5, Sia'-H6, Sia'-H9a), 4.14 (m, 1H, GalN-H4), 4.23 (dd, *J* = 7.1, 11.0 Hz, 1H, Gal-H6a), 4.27 (dd, *J* = 2.7, 12.5 Hz, 1H, Sia-H9b), 4.33 (dd, *J* = 3.2, 10.5 Hz, 1H, GalN-H3), 4.34 (dd, *J* = 2.7, 12.3 Hz, 1H, Sia'-H9b), 4.41 (dd, *J* = 6.5, 11.1 Hz, 1H, Gal-H6b), 4.66 (dd, *J* = 3.4, 10.1 Hz, 1H, Gal-H3), 4.83 (d, *J* = 8.2 Hz, 1H, GalN-H1), 4.83-4.88 (m, 2H, Sia-H4, Sia'-H4), 4.88 (d, *J* = 7.9 Hz, 1H, Gal-H1), 4.94 (d, *J* = 10.3 Hz, 1H, NH), 4.97 (A of AB, *J* = 12.1 Hz, 1H, PhC*H*$_2$), 5.07 (dd, *J* = 7.9, 10.0 Hz, 1H, Gal-H2), 5.18-5.21 (m, 2H, Gal-H4, NH), 5.28-5.34 (m, 3H, Sia-H7, Sia'-H7, PhC*H*$_2$), 5.36-5.41 (m, 2H, Sia-H8, Sia'-H8), 6.97 (d, *J* = 7.6 Hz, 1H, NH), 7.34-7.38, 7.44-7.47, 7.56-7.59, 8.05-8.06 (m, 10H, 2 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.5 ($SiMe_3$), 18.1 (CH_2Si), 20.7, 20.7, 20.7, 20.8, 20.9, 21.1, 21.3, 22.6, 23.1, 23.1 (10C, 12 CO*C*H_3), 37.5, 37.6 (Sia-C3, Sia'-C3), 48.9, 49.3 (Sia-C5, Sia'-C5), 52.8 (OCH_3), 55.0 (GalN-C2), 61.4 (Gal-C6), 62.2 (Sia-C9), 62.6 (GalN-C6), 62.9 (Sia'-C9), 66.7 (Sia-C7), 67.1 (3C, Sia-C8, Sia'-C7, OCH_2), 67.5 (2C, GalN-C4, Gal-C4), 68.4 (*C*H_2Ph), 68.8, 68.9, 69.0 (4C, Gal-C2, Sia-C4, Sia'-C4, Sia'-C8), 69.9 (Gal-C5), 70.4 (Gal-C3), 71.3 (GalN-C5), 72.0, 72.7 (Sia-C6, Sia'-C6), 77.6 (GalN-C3), 92.5 (CCl_3), 96.6 (Sia-C2), 98.5 (GalN-C1), 99.0 (Sia'-C2), 100.9 (Gal-C1), 128.4, 128.5, 128.6, 128.7, 129.5, 129.7, 133.3, 134.6 (12C, 2 C_6H_5), 161.6, 165.6, 167.3, 168.0, 169.4, 169.7, 169.9, 170.1, 170.1, 170.2, 170.2, 170.4, 170.5, 170.6, 170.8, 170.9 (16 CO); HRMS (FAB) Calcd for $C_{76}H_{104}Cl_3N_4O_{38}Si$ $[M+NH_4]^+$: 1813.5163; Found: 1813.5169.

12b : $[\alpha]_D$ -1.27 (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ -0.03 (s, 9H, $SiMe_3$), 0.82-0.98 (m, 2H, C*H*$_2$Si), 1.65 (t, *J* = 12.5 Hz, 1H, Sia-H3a), 1.82, 1.83 (2s, 6H, 2 $COCH_3$), 1.85 (m, 1H, Sia'-H3a), 1.92, 1.99, 2.06, 2.06, 2.08, 2.09, 2.13, 2.14, 2.15, 2.21 (10s, 30H, 10 $COCH_3$), 2.38 (dd, *J* = 4.5, 12.9 Hz, 1H, Sia'-H3b), 2.63 (dd, *J* = 4.6, 12.6 Hz, 1H, Sia-H3b), 3.39 (m, 1H, GalN-H5), 3.45-3.57 (m, 3H, GalN-H6a, Sia-H6, OC*H*$_2$-Ha), 3.65 (s, 1H, OH), 3.73-3.80 (m, 2H, GalN-H2, GalN-H6b), 3.83 (s, 3H, OCH_3), 3.91 (m, 1H, OC*H*$_2$-Hb), 3.97-4.04 (m, 2H, Sia-H5, Sia-H9a), 4.06-4.13 (m, 2H, Gal-H5, Sia'-H5), 4.18 (m, 1H, Sia'-H9a),

4.21 (dd, *J* = 3.3, 10.7 Hz, 1H, GalN-H3), 4.24 (m, 1H, GalN-H4), 4.27-4.35 (m, 2H, Sia-H9b, Sia'-H6), 4.40 (dd, *J* = 5.8, 11.0 Hz, 1H, Gal-H6a), 4.49 (dd, *J* = 6.9, 11.0 Hz, 1H, Gal-H6b), 4.67 (d, *J* = 8.1 Hz, 1H, GalN-H1), 4.68-4.74 (m, 2H, Gal-H3, Sia'-H9b), 4.87 (m, 1H, Sia-H4), 4.88 (d, *J* = 7.9 Hz, 1H, Gal-H1), 4.99 (d, *J* = 10.4 Hz, 1H, NH), 5.03 (A of AB, *J* = 12.1 Hz, 1H, PhC*H*$_2$), 5.11 (dd, *J* = 7.9, 10.1 Hz, 1H, Gal-H2), 5.19 (d, *J* = 3.3 Hz, 1H, Gal-H4), 5.26 (m, 1H, Sia'-H4), 5.32 (m, 1H, Sia-H8), 5.37 (B of AB, *J* = 12.1 Hz, 1H, PhC*H*$_2$), 5.37-5.45 (m, 2H, Sia-H7, Sia'-H8), 5.49 (m, 1H, Sia'-H7), 6.70-6.72 (m, 2H, 2 NH), 7.34-7.42, 7.46-7.51, 7.57-7.61, 8.04-8.07 (m, 10H, 2 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.4 ($SiMe_3$), 18.1 (CH_2Si), 20.5, 20.7, 20.7, 20.8, 20.8, 20.9, 21.1, 21.2, 21.4, 22.6, 22.9, 23.1 (12 CO*C*H$_3$), 37.5, 37.7 (Sia-C3, Sia'-C3), 48.9, 49.0 (Sia-C5, Sia'-C5), 52.8 (OCH_3), 54.5 (GalN-C2), 61.8 (Gal-C6), 62.1 (Sia-C9), 62.3 (2C, GalN-C6, Sia'-C9), 66.8, 66.9 (GalN-C4, Sia'-C8), 67.3 (OCH_2), 67.5, 67.7 (Gal-C4, Sia-C8), 68.2 (Sia'-C7), 68.5 (*C*H$_2$Ph), 69.0, 69.0 (Sia-C4, Sia'-C4), 70.1 (Sia-C7), 70.9 (Gal-C2), 71.1 (2C, GalN-C5, Gal-C5), 71.4 (Gal-C3), 71.5 (Sia'-C6), 72.1 (Sia-C6), 72.2 (GalN-C3), 92.6 (CCl_3), 96.7 (Sia-C2), 98.4 (GalN-C1), 99.0 (Sia'-C2), 100.9 (Gal-C1), 128.6, 128.6, 128.7, 129.6, 129.8, 129.8, 133.4, 134.6 (12C, 2 C_6H_5), 161.6, 166.1, 167.4, 167.4, 169.4, 170.2, 170.3, 170.3, 170.5, 170.5, 170.5, 170.6, 170.8, 171.3 (16C, 16 CO); HRMS (FAB) Calcd for $C_{76}H_{104}Cl_3N_4O_{38}Si$ $[M+NH_4]^+$: 1813.5163; Found: 1813.5171.

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)]-(2→6)-2-acetamido-2-deoxy-β-D-galactopyranoside (13). A solution of **12**a (70.0 mg, 38.9 μmol), tributyltin hydride (41.2 μl, 156 μmol), and AIBN (2 mg) in dry benzene (2 mL) was stirred for 1 h at r.t. under argon, then heated under reflux for 2.5 h, cooled, and concentrated under reduced pressure. The residue was dissolved in MeCN (20 mL) and washed with hexane (2 × 5 mL). The acetonitrile was evaporated and the residue purified by column chromatography on silica gel (DCM/MeOH 10:1) to give **13** (48.1 mg, 73%) as a colorless foam.

$[\alpha]_D$ -6.8 (*c* 0.75, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ -0.01 (s, 9H, $SiMe_3$), 0.86-0.97 (m, 2H, C*H*$_2$Si), 1.64 (m, 1H, Sia-H3a), 1.83, 1.87 (2s, 6H, 2 $COCH_3$), 1.92 (t, *J* = 12.6 Hz, 1H, Sia'-H3a), 1.97, 1.98, 2.00, 2.03, 2.06, 2.06, 2.09, 2.11, 2.12, 2.14, 2.21 (11s, 33H, 11 $COCH_3$), 2.57 (dd, *J* = 4.6, 12.8 Hz, 1H, Sia'-H3b), 2.62 (dd, *J* = 4.7, 12.6 Hz, 1H, Sia-H3b), 2.63 (s, 1H, OH), 3.39 (m, 1H, GalN-H2), 3.47 (dd, *J* = 2.6, 10.7 Hz, 1H, Sia-H6), 3.53 (m,

1H, OCH_2-Ha), 3.60 (m, 1H, GalN-H5), 3.67 (dd, *J* = 7.2, 9.5 Hz, 1H, GalN-H6a), 3.80 (s, 3H, OCH_3), 3.89 (dd, *J* = 5.6, 9.5 Hz, 1H, GalN-H6b), 3.96-4.14 (m, 8H, GalN-H4, Gal-H5, Sia-H5, Sia-H9a, Sia'-H5, Sia'-H6, Sia'-H9a, OCH_2-Hb), 4.23 (dd, *J* = 7.4, 11.0 Hz, 1H, Gal-H6a), 4.33 (dd, *J* = 2.6, 12.5 Hz, 2H, Sia-H9b, Sia'-H9b), 4.41 (dd, *J* = 6.3, 11.0 Hz, 1H, Gal-H6b), 4.44 (dd, *J* = 3.1, 10.7 Hz, 1H, GalN-H3), 4.67 (dd, *J* = 3.3, 10.1 Hz, 1H, Gal-H3), 4.81 (d, *J* = 8.0 Hz, 1H, Gal-H1), 4.83-4.87 (m, 2H, Sia-H4, Sia'-H4), 4.94 (d, *J* = 8.3 Hz, 1H, GalN-H1), 4.94 (d, *J* = 10.4 Hz, 1H, NH), 4.97 (A of AB, *J* = 12.1 Hz, 1H, PhCH_2), 5.06 (dd, *J* = 8.0, 10.0 Hz, 1H, Gal-H2), 5.16 (d, *J* = 10.0 Hz, 1H, NH), 5.18 (d, *J* = 3.3 Hz, 1H, Gal-H4), 5.29-5.34 (m, 3H, Sia-H7, Sia'-H7, PhCH_2), 5.37 (m, 1H, Sia-H8), 5.49 (m, 1H, Sia'-H8), 5.74 (d, *J* = 7.3 Hz, 1H, NH), 7.35-7.39, 7.44-7.47, 7.56-7.59, 8.05-8.07 (m, 10H, 2 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.4 ($SiMe_3$), 17.9 (CH_2Si), 20.7, 20.7, 20.8, 20.8, 20.9, 20.9, 21.0, 21.4, 23.1, 23.2, 23.6 (13C, 13 CO*C*H_3), 37.5 (2C, Sia-C3, Sia'-C3), 48.9, 49.5 (Sia-C5, Sia'-C5), 52.9 (OCH_3), 54.3 (GalN-C2), 61.4 (Gal-C6), 62.3, 62.3 (Sia-C9, Sia'-C9), 62.9 (GalN-C6), 66.6 (OCH_2), 66.7 (GalN-C4), 67.0 (Gal-C4), 67.1, 67.5, 67.6 (4C, Sia-C7, Sia-C8, Sia'-C7, Sia'-C8), 68.4 (*C*H_2Ph), 69.0, 69.1 (Sia-C4, Sia'-C4), 69.1 (Gal-C2), 69.9 (Gal-C5), 70.4 (Gal-C3), 71.3 (GalN-C5), 71.9, 72.7 (Sia-C6, Sia'-C6), 78.3 (GalN-C3), 96.7 (Sia-C2), 98.7 (GalN-C1), 98.9 (Sia'-C2), 100.9 (Gal-C1), 128.4, 128.6, 128.6, 128.8, 129.6, 129.7, 133.3, 134.7 (12C, 2 C_6H_5), 165.7, 167.4, 168.1, 169.5, 169.6, 169.9, 170.1, 170.1, 170.2, 170.3, 170.4, 170.6, 170.7, 170.9 (16C, 16 CO); MS (ESI) Calcd for $C_{76}H_{103}N_3NaO_{38}Si$ $[M+Na]^+$: 1716.6; Found: 1716.7.

2-(Trimethylsilyl)ethyl (sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-(β-D-galactopyranosyl)-(1→3)-[(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)]-(2→6)-2-acetamido-2-deoxy-β-D-galactopyranoside (2). A solution of **13** (42.0 mg, 24.8 μmol) in MeOH (3 mL) was treated with freshly prepared 1 M NaOMe/MeOH (0.2 mL) for 20 h under argon at r.t. Then water (0.5 mL) was added and the mixture was stirred for another 6 h at r.t. The solution was concentrated *in vacuo* and the residue purified by reversed-phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **2** (21.0 mg, 76%) as a colorless solid after a final lyophilization from water.

$[\alpha]_D$ -6.0 (*c* 0.60, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.00 (s, 9H, $SiMe_3$), 0.86 (m, 1H, CH_2Si-Ha), 0.97 (ddd, *J* = 7.2, 10.9, 13.7 Hz, 1H, CH_2Si-Hb), 1.66 (t, *J* = 12.2 Hz, 1H, Sia'-H3a), 1.77 (t, *J* = 12.1 Hz, 1H, Sia-H3a), 1.99, 2.01, 2.02 (3s, 9H, 3 $COCH_3$), 2.71 (dd, *J* =

4.6, 12.5 Hz, 1H, Sia'-H3b), 2.74 (dd, *J* = 4.7, 12.8 Hz, 1H, Sia-H3b), 3.52 (dd, *J* = 7.9, 9.8 Hz, 1H, Gal-H2), 3.55-3.73 (m, 16H, GalN-H5, GalN-H6, Gal-H5, Gal-H6, Sia-H4, Sia-H6, Sia-H7, Sia-H8, Sia-H9a, Sia'-H4, Sia'-H6, Sia'-H7, Sia'-H8, Sia'-H9a), 3.74 (m, 1 H, OCH_2-Ha), 3.79-3.88 (m, 6H, GalN-H3, Sia-H5, Sia'-H5, 3 NH), 3.89 (dd, *J* = 2.4, 10.8 Hz, 1H, Sia-H9b), 3.91-3.93 (m, 2H, Gal-H4, Sia'-H9b), 3.98 (m, 1H, GalN-H2), 4.03 (m, 1H, OCH_2-Hb), 4.05 (dd, *J* = 3.1, 9.8 Hz, 1H, Gal-H3), 4.18 (m, *J* = 3.2 Hz, 1H, GalN-H4), 4.88 (d, *J* = 7.8 Hz, 1H, Gal-H1), 4.83 (d, *J* = 8.6 Hz, 1H, GalN-H1); ^{13}C NMR (125 MHz, D_2O): δ -1.7 ($SiMe_3$), 17.9 (CH_2Si), 22.7, 23.1 (3C, 3 COCH_3), 40.4, 41.0 (Sia-C3, Sia'-C3), 51.7, 52.4, 52.6 (GalN-C2, Sia-C5, Sia'-C5), 61.7 (Gal-C6), 63.2 (GalN-C6), 63.3, 64.2 (Sia-C9, Sia'-C9), 68.6 (Gal-C4), 68.8, 68.9 (3C, GalN-C4, Sia-C7, Sia'-C7), 69.1 (OCH_2), 69.2 (2C, Sia-C4, Sia'-C4), 69.7 (Gal-C2), 72.4, 72.5 (Sia-C8, Sia'-C8), 73.4, 73.5 (Sia-C6, Sia'-C6), 73.9 (Gal-C5), 75.4 (GalN-C5), 76.3 (Gal-C3), 81.2 (GalN-C3), 99.6, 100.1 (Sia-C2, Sia'-C2), 101.4 (GalN-C1), 105.4 (Gal-C1), 172.8, 173.1, 173.9 (5C, 5 CO); HRMS (FAB) Calcd for $C_{41}H_{72}N_3O_{27}Si$ $[M+H]^+$: 1066.4122; Found: 1066.4105.

2-(Trimethylsilyl)ethyl (β-D-galactopyranosyl)-(1→3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (15). A solution of **14**[S23] (550 mg, 0.53 mmol) in MeOH (20 mL) was treated with 1 M NaOMe/MeOH (2 mL) at r.t. under argon for 2 h. The reaction mixture was neutralized with Amberlite IRC 50 ion-exchange resin and filtered through a pad of Celite. The Celite was washed with methanol (3 × 5 mL), and the combined filtrates were evaporated to dryness. The residue was purified by column chromatography on silica gel (DCM/MeOH 15:1) to give **15** (280 mg, 85%) as a colorless foam.

$[\alpha]_D$ -7.7 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.03 (s, 9H, $SiMe_3$), 0.97 (t, *J* = 8.4 Hz, 2H, CH_2Si), 3.42 (m, 1H, Gal-H5), 3.43 (dd, *J* = 3.4, 9.8 Hz, 1H, Gal'-H3), 3.54-3.63 (m, 3H, Gal-H2, Gal'-H2, OCH_2-Ha), 3.64-3.71 (m, 5H, Gal-H6, Gal'-H5, Gal'-H6), 3.76 (dd, *J* = 3.4, 9.7 Hz, 1H, Gal-H3), 3.78 (d, *J* = 3.3 Hz, 1H, Gal'-H4), 4.00 (dd, *J* = 8.6, 17.4 Hz, 1H, OCH_2-Hb), 4.02 (d, *J* = 3.4 Hz, 1H, Gal-H4), 4.37 (d, *J* = 7.8 Hz, 1H, Gal-H1), 4.54, 4.57 (A, B of AB, *J* = 11.9 Hz, 2H, PhCH_2), 4.58 (d, *J* = 7.8 Hz, 1H, Gal'-H1), 4.78, 4.84 (A, B of AB, *J* = 10.7 Hz, 2H, PhCH_2), 7.21-7.33, 7.40 (m, 10H, 2 C_6H_5); ^{13}C-NMR (125 MHz, CD_3OD): δ -1.5 ($SiMe_3$), 19.3 (CH_2Si), 62.4 (Gal'-C6), 68.2 (OCH_2), 70.2 (Gal'-C4), 70.5 (Gal-C4), 70.7 (Gal-C6), 72.7 (Gal'-C3), 74.2 (PhCH_2), 74.6 (Gal'-C2), 75.8 (PhCH_2), 76.6 (2C, Gal-C5, Gal'-C5), 80.3 (Gal-C2), 81.8 (Gal-C3), 104.3 (Gal-C1), 105.9 (Gal'-C1), 128.4, 128.6, 128.7, 129.0, 129.2, 129.3, 139.6, 140.2 (12C, 2 C_6H_5); HRMS (FAB) Calcd for $C_{31}H_{46}NaO_{11}Si$ $[M+Na]^+$: 645.2707; Found: 645.2711.

2-(Trimethylsilyl)ethyl (6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (16). To a solution of **15** (95.0 mg, 0.15 mmol) in NEt_3 (1.5 mL) and MeCN (5.0 mL) at -40°C under argon, a solution of benzoyl cyanide (18.0 mg, 0.14 mmol) in MeCN (1 mL) was added drop-wise during 15 min. The reaction mixture was stirred at -40°C for 2 h and then quenched by adding MeOH (0.5 mL). The solution was concentrated under reduced pressure and the residue purified by column chromatography on silica gel (DCM/MeOH 40:1) to afford **16** (73.4 mg, 66%) as a colorless foam.

$[\alpha]_D$ -0.3 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.03 (s, 9H, $SiMe_3$), 0.96 (m, 2H, C*H*$_2$Si), 3.42-3.46 (m, 2H, Gal-H5, Gal-H6a), 3.51 (dd, *J* = 3.4, 9.7 Hz, 1H, Gal'-H3), 3.55-3.64 (m, 5H, Gal-H2, Gal-H3, Gal-H6b, Gal'-H2, OC*H*$_2$-Ha), 3.83 (dd, *J* = 4.2, 8.5 Hz, 1H, Gal'-H5), 3.86 (d, *J* = 3.4 Hz, 1H, Gal'-H4), 3.96 (m, 1H, OC*H*$_2$-Hb), 3.98 (d, *J* = 3.6 Hz, 1H, Gal-H4), 4.26 (d, *J* = 7.6 Hz, 1H, Gal-H1), 4.37 (dd, *J* = 4.2, 11.4 Hz, 1H, Gal'-H6a), 4.43, 4.46 (A, B of AB, *J* = 12.0 Hz, 2H, PhC*H*$_2$), 4.57 (d, *J* = 7.8 Hz, 1H, Gal'-H1), 4.63 (dd, *J* = 8.5, 11.4 Hz, 1H, Gal'-H6b), 4.76, 4.84 (A, B of AB, *J* = 10.6 Hz, 2H, PhC*H*$_2$), 7.21-7.32, 7.40-7.46, 7.54-7.58, 7.99-8.01 (m, 15H, 3 C_6H_5); ^{13}C-NMR (125 MHz, $CDCl_3$): δ -1.5 ($SiMe_3$), 19.3 (CH_2Si), 65.0 (Gal'-C6), 68.2 (OCH_2), 70.1, 70.6 (Gal-C4, Gal'-C4), 70.9 (Gal-C6), 72.4 (2C, Gal'-C2, Gal'-C3), 74.0 (Gal'-C5), 74.1 (Ph*C*H$_2$), 74.6 (Gal-C5), 75.9 (Ph*C*H$_2$), 80.0 (Gal-C2), 82.7 (Gal-C3), 104.2 (Gal-C1), 105.9 (Gal'-C1), 128.4, 128.5, 128.6, 129.0, 129.2, 129.3, 129.6, 129.7, 130.5, 130.7, 131.1, 131.5, 132.2, 134.3, 139.6, 140.1 (18C, 3 C_6H_5), 167.6 (CO); HRMS (FAB) Calcd for $C_{38}H_{54}NO_{12}Si$ $[M+NH_4]^+$: 744.3415; Found: 744.3402.

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (17). To a solution of **16** (170 mg, 0.23 mmol) and donor **11** (244 mg, 0.47 mmol) in MeCN (6 mL) was added activated powdered molecular sieves 3Å (2.0 g). The reaction mixture was stirred at r.t. under argon for 6 h and then cooled to -30°C. After the addition of NIS (210 mg, 0.94 mmol) and TfOH (8.2 μL, 0.09 mmol), stirring was continued for 16 h at -30°C. Then the mixture was diluted with DCM (10 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3 × 10 mL), and the combined filtrates were washed with 20% aqueous $Na_2S_2O_3$ (30 mL), saturated aqueous $KHCO_3$ (2 × 20 mL) and H_2O (20 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (DCM/MeOH 60:1) to afford **17** (150 mg, 54%) as a colorless foam.

$[\alpha]_D$ +1.1 (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 1.01 (m, 2H, CH_2Si), 1.89, 2.00, 2.04, 2.06, 2.10 (5s, 15H, 5 $COCH_3$), 2.10 (m, 1H, Sia-H3a), 2.66-2.69 (m, 2H, Sia-H3b, OH), 2.87 (br s, 1H, OH), 3.52-3.59 (m, 3H, Gal-H5, Gal-H6a, OCH_2-Ha), 3.65-3.68 (m, 2H, Gal-H2, Gal-H6b), 3.71 (dd, *J* = 3.3, 11.6 Hz, 1H, Gal-H3), 3.76 (m, 1H, Gal'-H2), 3.79 (s, 3H, OCH_3), 3.86 (m, 1H, Gal'-H5), 3.98 (dd, *J* = 5.6, 12.5 Hz, 1H, Sia-H9a), 4.00-4.05 (m, 4H, Gal-H4, Gal'-H4, Sia-H5, OCH_2-Hb), 4.10 (dd, *J* = 1.9, 10.8 Hz, 1H, Sia-H6), 4.11 (dd, *J* = 3.5, 9.5 Hz, 1H, Gal'-H3), 4.23 (dd, *J* = 2.6, 12.5 Hz, 1H, Sia-H9b), 4.34 (d, *J* = 7.6 Hz, 1H, Gal-H1), 4.46, 4.51 (A, B of AB, *J* = 12.0 Hz, 2H, PhCH_2), 4.53 (m, 1H, Gal'-H6a), 4.61 (dd, *J* = 4.8, 11.6 Hz, 1H, Gal'-H6b), 4.63 (d, *J* = 7.7 Hz, 1H, Gal'-H1), 4.83 (m, 2H, PhCH_2), 4.97 (ddd, *J* = 4.6, 10.3, 12.0 Hz, 1H, Sia-H4), 5.23 (d, *J* = 9.7 Hz, 1H, NH), 5.31 (dd, *J* = 1.8, 8.6 Hz, 1H, Sia-H7), 5.36 (ddd, *J* = 2.6, 5.5, 8.4 Hz, 1H, Sia-H8), 7.23-7.33, 7.38-7.42, 7.52-7.55, 8.01-8.04 (m, 15H, 3 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.5 ($SiMe_3$), 18.5 (CH_2Si), 20.7, 20.7, 20.8, 21.2, 23.2 (5 CO*C*H_3), 37.3 (Sia-C3), 49.6 (Sia-C5), 53.2 (OCH_3), 62.3 (Sia-C9), 63.4 (Gal'-C6), 67.0 (Sia-C7), 67.2 (OCH_2), 67.8 (Gal'-C2), 68.2 (Sia-C8), 68.6 (Sia-C4), 68.8, 69.4 (Gal-C4, Gal'-C4), 70.0 (Gal-C6), 72.2 (Sia-C6), 72.7 (Gal'-C5), 73.3 (Gal-C5), 73.5 (Ph*C*H_2), 74.9 (Ph*C*H_2), 76.1 (2C, Gal-C2, Gal'-C3), 84.4 (Gal-C3), 97.8 (Sia-C2), 102.9 (Gal-C1), 103.8 (Gal'-C1), 127.6, 128.2, 128.2, 128.4, 128.5, 129.7, 129.7, 138.2, 138.8 (18C, 3 C_6H_5), 168.3, 170.2 (7C, 7 CO); HRMS (FAB) Calcd for $C_{58}H_{77}NNaO_{24}Si$ $[M+Na]^+$: 1222.4502; Found: 1222.4511.

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α**-D-*galacto*-2-nonulopyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-**β**-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-**β**-D-galactopyranoside (18)**. To a stirred solution of **17** (100 mg, 0.083 mmol) in pyridine (1.0 mL) at 0°C was added acetic anhydride (0.5 mL). The mixture was stirred for 16 h at r.t. under argon and then quenched by adding MeOH (2 mL). The solution was concentrated *in vacuo* and co-evaporated with toluene (3 × 10 mL). The residue was purified by silica gel chromatography (toluene/EtOAc 1:1) to give **18** (88.0 mg, 80%) as a colorless foam.

$[\alpha]_D$ -13.2 (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 1.03 (m, 2H, CH_2Si), 1.68 (t, *J* = 12.5 Hz, 1H, Sia-H3a), 1.83, 1.90, 1.98, 1.99, 2.00, 2.10, 2.11, 2.12 (8s, 24H, 8 $COCH_3$), 2.58 (dd, *J* = 4.6, 12.7 Hz, 1H, Sia-H3b), 3.47-3.58 (m, 3H, Gal-H6, OCH_2-Ha), 3.61 (dd, *J* = 2.6, 10.9 Hz, 1H, Sia-H6), 3.64 (dd, *J* = 7.9, 9.6 Hz, 1H, Gal-H2), 3.88 (dd, *J* = 3.6, 9.6 Hz, 1H, Gal-H3), 3.72 (m, 1H, Gal-H5), 3.79 (s, 3H, OCH_3), 3.94 (dd, *J* = 5.9, 12.5 Hz, 1H, Sia-H9a), 4.02-4.04 (m, 3H, Gal'-H5, Sia-H5, OCH_2-Hb), 4.18 (dd, *J* = 7.8, 11.0

Hz, 1H, Gal'-H6a), 4.30 (dd, *J* = 2.6, 12.5 Hz, 1H, Sia-H9b), 4.41 (m, 1H, Gal'-H6b), 4.42 (d, *J* = 8.0 Hz, 1H, Gal-H1), 4.48, 4.51 (A, B of AB, *J* = 11.8 Hz, 2H, PhC*H*$_2$), 4.59 (dd, *J* = 3.2, 10.2 Hz, 1H, Gal'-H3), 4.75, 4.83 (A, B of AB, *J* = 11.0 Hz, 2H, PhC*H*$_2$), 4.84 (d, *J* = 7.7 Hz, 1H, Gal'-H1), 4.88 (m, 1H, Sia-H4), 5.05 (d, *J* = 3.2 Hz, 1H, Gal'-H4), 5.11 (dd, *J* = 7.9, 10.1 Hz, 1H, Gal'-H2), 5.21 (d, *J* = 10.2 Hz, 1H, NH), 5.34 (dd, *J* = 2.6, 8.9 Hz, 1H, Sia-H7), 5.46 (d, *J* = 3.6 Hz, 1H, Gal-H4), 5.50 (ddd, *J* = 2.7, 5.9, 8.8 Hz, 1H, Sia-H8), 7.24-7.28, 7.32-7.35, 7.40-7.43, 7.52-7.56, 8.03-8.05 (m, 15H, 3 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.6 ($SiMe_3$), 18.4 (CH_2Si), 20.6, 20.7, 20.7, 20.7, 20.9 (8C, 8 CO*C*H_3), 37.4 (Sia-C3), 49.0 (Sia-C5), 52.9 (OCH_3), 67.1 (Sia-C7), 67.2 (Gal'-C4), 67.7 (OCH_2), 67.8 (Sia-C8), 69.2 (Sia-C4), 69.3 (Gal-C4), 69.4 (Gal-C6), 70.1 (Gal'-C2), 70.4 (Gal'-C5), 71.5 (Gal'-C3), 72.0 (Sia-C6) 72.9 (Gal-C5), 73.6 (Ph*C*H_2), 75.1 (Ph*C*H_2), 78.6 (Gal-C2), 80.2 (Gal-C3), 96.6 (Sia-C2), 100.7 (Gal'-C1), 103.1 (Gal-C1), 127.4, 127.6, 127.7, 127.7, 128.1, 128.1, 128.2, 128.3, 128.4, 128.8, 129.0, 129.6, 129.8, 130.8, 133.0, 137.8, 137.9, 138.9 (18C, 3C_6H_5), 165.6, 167.9, 169.6, 169.6, 170.2, 170.2, 170.3, 170.5, 170.5, 170.8 (10CO); MS (ESI) Calcd for $C_{64}H_{83}NNaO_{27}Si$ $[M+Na]^+$: 1348.5; Found: 1348.3.

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-β-D-galactopyranoside (19). A solution of compound **18** (81.0 mg, 0.061 mmol) in ethanol (4.0 mL) and acetic acid (0.8 mL) was hydrogenated (1 bar H_2) in the presence of 10% Pd-C (120 mg) for 4 h at 45-50°C. The reaction mixture was filtered through a pad of Celite, which was washed with DCM (5 mL). The combined filtrates were concentrated and the residue purified by silica gel chromatography (DCM/MeOH 50:1) to afford **19** (53.5 mg, 76%) as a colorless foam.

$[\alpha]_D$ -2.8 (*c* 1.00, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 1.06 (m, 2H, C*H*$_2$Si), 1.71 (t, *J* = 12.4 Hz, 1H, Sia-H3a), 1.84, 1.99, 2.04, 2.07, 2.13, 2.21 (7s, 24H, 8 $COCH_3$), 2.57 (dd, *J* = 4.6, 12.6 Hz, 1H, Sia-H3b), 2.94 (br s, 1H, OH), 3.44 (m, 1H, Gal-H6a), 3.55-3.60 (m, 3H, Gal-H5, Gal-H6b, OC*H*$_2$-Ha), 3.62 (dd, *J* = 2.7, 10.6 Hz, 1H, Sia-H6), 3.73 (s, 3H, OCH_3), 3.78 (m, 2H, Gal-H2, Gal-H3), 3.93 (dd, *J* = 6.7, 12.2 Hz, 1H, Sia-H9a), 3.98 (m, 2H, Gal'-H5, OC*H*$_2$-Hb), 4.06 (m, 1H, Sia-H5), 4.16 (dd, *J* = 7.2, 11.2 Hz, 1H, Gal'-H6a), 4.32 (d, *J* = 7.6 Hz, 1H, Gal-H1), 4.32-4.36 (m, 2H, Gal'-H6b, Sia-H9b), 4.58 (dd, *J* = 3.4, 10.2 Hz, 1H, Gal'-H3), 4.85 (dt, *J* = 4.6, 12.0 Hz, 1H, Sia-H4), 4.91 (d, *J* = 7.9 Hz, 1H, Gal'-H1), 5.03 (m, 2H, Gal'-H2, Gal'-H4), 5.20 (d, *J* = 10.3 Hz, 1H, NH), 5.28 (d, *J* = 3.4 Hz, 1H, Gal-H4), 5.32 (dd, *J* = 2.7, 9.3 Hz, 1H, Sia-H7), 5.62 (ddd, *J* = 2.7, 6.7, 9.3 Hz, 1H,

Sia-H8), 7.40-7.44, 7.53-7.56, 8.01-8.03 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.5 ($SiMe_3$), 18.2 (CH_2Si), 20.7, 20.7, 20.8, 20.8, 20.9 (8C, 8 $COCH_3$), 37.4 (Sia-C3), 48.9 (Sia-C5), 53.0 (OCH_3), 60.0 (Gal-C6), 61.1 (Gal'-C6), 62.9 (Sia-C9), 67.3, 67.3 (Gal'-C4, Sia-C7), 67.4 (Sia-C8), 67.7 (OCH_2), 69.2 (Sia-C4), 69.3 (Gal-C4), 69.8 (Gal'-C2), 70.6, 70.7, 71.0 (Gal-C2, Gal'-C3, Gal'-C5), 71.9 (Sia-C6) 73.2 (Gal-C5), 79.7 (Gal-C3), 96.7 (Sia-C2), 101.2 (Gal'-C1), 102.5 (Gal-C1), 128.2, 128.3, 129.6, 129.7, 133.1 (6C, C_6H_5), 165.7, 165.8, 167.9, 169.8, 170.1, 170.3, 170.6, 170.9, 171.0, 172.7 (10 CO); HRMS (FAB) Calcd for $C_{50}H_{72}NO_{27}Si$ $[M+H]^+$: 1146.4061; Found: 1146.4102.

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α**-D-*galacto*-2-nonulopyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-**β**-D-galactopyranosyl)-(1→3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-**α**-and **β**-D-*galacto*-2-nonulopyranosynate)]-(2→6)-4-*O*-acetyl-**β**-D-galactopyranoside (20).** To a solution of **19** (61.3 mg, 0.05 mmol) and donor **11** (70.0 mg, 0.13 mmol) in MeCN (5 mL) was added activated powdered molecular sieves 3Å (2.0 g). The reaction mixture was stirred at r.t. under argon for 6 h and then cooled to -40°C. After the addition of NIS (60.0 mg, 0.27 mmol) and TfOH (2.4 μL, 0.03 mmol), stirring was continued for 16 h at -40°C. Then the mixture was diluted with DCM (10 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3 × 10 mL), and the combined filtrates were washed with 20% aqueous $Na_2S_2O_3$ (20 mL), saturated aqueous $KHCO_3$ (2 × 10 mL) and H_2O (10 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH 35:1) to afford **20**a (33.0 mg, 38%) and **20**b (10.0 mg, 12%) as colorless foams.

20a : $[\alpha]_D$ -14.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 1.07 (m, 2H, C*H*$_2$Si), 1.69 (t, *J* = 12.3 Hz, 1H, Sia-H3a), 1.83, 1.85 (2s, 6H, 2 $COCH_3$), 1.90 (t, *J* = 12.6 Hz, 1H, Sia'-H3a), 1.98, 1.99, 2.00, 2.03, 2.06, 2.07, 2.08, 2.10, 2.12 (9s, 33H, 11 $COCH_3$), 2.55 (m, 2H, Sia-H3b, Sia'-H3b), 3.18 (br s, 1H, OH), 3.28 (dd, *J* = 6.3, 9.6 Hz, 1H, Gal-H6a), 3.46-3.67 (m, 3H, Sia-H6, Sia'-H6, OC*H*$_2$-Ha), 3.68-3.78 (m, 2H, Gal-H2, Gal-H5), 3.72, 3.75 (2s, 6H, 2 OCH_3), 3.79-3.84 (m, 2H, Gal-H3, Gal-H6b), 3.91 (dd, *J* = 7.2, 12.2 Hz, 1H, Sia-H9a), 3.96 (t, *J* = 7.0 Hz, 1H, Gal'-H5), 4.00-4.04 (m, 3H, Sia-H5, Sia'-H5, OC*H*$_2$-Hb), 4.07 (dd, *J* = 5.3, 11.9 Hz, 1H, Sia'-H9a), 4.18 (m, 1H, Gal'-H6a), 4.27 (m, 1H, Sia'-H9b), 4.28 (d, *J* = 7.6 Hz, 1H, Gal-H1), 4.34-4.40 (m, 2H, Gal'-H6b, Sia-H9b), 4.55 (dd, *J* = 3.4, 10.0 Hz, 1H, Gal'-H3), 4.83 (m, 2H, Sia-H4, Sia'-H4), 4.97-5.01 (m, 2H, Gal'-H2, Gal'-H4), 5.05 (d, *J* = 8.0 Hz, 1H, Gal'-H1), 5.12 (d, *J* = 10.2 Hz, 1H, NH), 5.18 (d, *J* = 8.0

Hz, 1H, NH), 5.27-5.34 (m, 3H, Sia-H7, Sia'-H7, Sia'-H8), 5.39 (d, *J* = 3.5 Hz, 1H, Gal-H4), 5.61 (ddd, *J* = 2.8, 7.3, 9.5 Hz, 1H, Sia-H8), 7.41-7.42, 7.53-7.56, 8.00-8.02 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.5 ($SiMe_3$), 18.2 (CH_2Si), 20.7, 20.7, 20.8, 20.9, 20.9 (13C, 13 CO*C*H_3), 37.7, 38.7 (Sia-C3, Sia'-C3), 49.0, 49.4 (Sia-C5, Sia'-C5), 52.8, 53.0 (2 OCH_3), 61.4 (Gal'-C6), 62.3 (Sia'-C9), 62.9, 63.1 (Gal-C6, Sia-C9), 67.3, 67.4 (3C, Gal'-C4, Sia-C7, Sia-C8), 67.5 (OCH_2), 68.9, 69.3, 69.3, 70.6 (5C, Gal-C4, Sia-C4, Sia'-C4, Sia'-C7, Sia'-C8), 71.3, 71.3, 72.0, 72.1, 72.6 (7C, Gal-C2, Gal-C5, Gal'-C2, Gal'-C3, Gal'-C5, Sia-C6, Sia'-C6), 77.8 (Gal-C3), 96.7, 98.7 (Sia-C2, Sia'-C2), 100.1 (Gal'-C1), 102.4 (Gal-C1), 128.3, 129.7, 133.1 (6C, C_6H_5), 165.7, 167.7, 169.6, 169.7, 170.0, 170.1, 170.2, 170.3, 170.6, 170.9, 171.2 (16C, 16 CO); HRMS (FAB) Calcd for $C_{70}H_{102}N_3O_{39}Si$ $[M+NH_4]^+$: 1636.5860; Found: 1636.5869.

20b : $[\alpha]_D$ -12.7 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 1.04 (m, 2H, C*H*$_2$Si), 1.72 (m, 2H, Sia-H3a, Sia'-H3a), 1.83, 1.92, 1.99, 2.01, 2.03, 2.04, 2.07, 2.08, 2.12, 2.13, 2.21, 2.22 (13s, 39H, 13 $COCH_3$), 2.40 (dd, *J* = 5.0, 12.8 Hz, 1H, Sia'-H3b), 2.57 (dd, *J* = 4.6, 12.6 Hz, 1H, Sia-H3b), 3.07 (br s, 1H, OH), 3.30 (t, *J* = 9.3 Hz, 1H, Gal-H6a), 3.40 (dd, *J* = 4.6, 8.7 Hz, 1H, Gal-H6b), 3.57 (m, 1H, OC*H*$_2$-Ha), 3.62 (dd, *J* = 2.7, 10.7 Hz, 1H, Sia-H6), 3.71 (dd, *J* = 2.5, 10.6 Hz, 1H, Sia'-H6), 3.73-3.83 (m, 3H, Gal-H2, Gal-H3, Gal-H5), 3.76, 3.78 (2s, 6H, 2 OCH_3), 3.92 (dd, *J* = 7.2, 12.2 Hz, 1H, Sia-H9a), 3.95-3.99 (m, 2H, Gal'-H5, OC*H*$_2$-Hb), 4.02-4.06 (m, 2H, Sia-H5, Sia'-H9a), 4.09-4.21 (m, 2H, Gal'-H6a, Sia'-H5), 4.31 (d, *J* = 7.6 Hz, 1H, Gal-H1), 4.37 (dd, *J* = 2.8, 12.2 Hz, 1H, Sia-H9b), 4.45 (dd, *J* = 5.6, 10.9 Hz, 1H, Gal'-H6b), 4.56 (dd, *J* = 3.3, 10.2 Hz, 1H, Gal'-H3), 4.73 (dd, *J* = 2.3, 12.3 Hz, 1H, Sia'-H9b), 4.84 (m, 1H, Sia-H4), 4.98-5.00 (m, 3H, Gal'-H1, Gal'-H2, Sia'-H8), 5.05 (d, *J* = 3.2 Hz, 1H, Gal'-H4), 5.10 (d, *J* = 10.3 Hz, 1H, NH), 5.30 (dd, *J* = 2.7, 9.1 Hz, 1H, Sia-H7), 5.33-5.35 (m, 2H, Sia'-H4, Sia'-H7), 5.59 (d, *J* = 3.1 Hz, 1H, Gal-H4), 5.63 (ddd, *J* = 2.8, 7.2, 9.5 Hz, 1H, Sia-H8), 6.22 (d, *J* = 10.2 Hz, 1H, NH), 7.38-7.41, 7.51-7.54, 7.97-7.98 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ -1.3 ($SiMe_3$), 18.4 (CH_2Si), 20.8, 20.9, 20.9, 21.1, 21.4, 21.5 (13C, 13 CO*C*H_3), 37.2, 37.4 (Sia-C3, Sia'-C3), 47.8, 49.1 (Sia-C5, Sia'-C5), 53.0, 53.1 (2 OCH_3), 60.2 (Gal-C6), 61.3 (Gal'-C6), 62.6 (Sia'-C9), 63.2 (Sia-C9), 67.5 (Gal'-C4), 67.5 (Sia-C7), 67.7 (OCH_2), 68.2 (Sia'-C7), 68.4 (Sia-C8), 69.0, 69.0 (Gal-C4, Sia'-C4), 69.4 (Sia-C4), 70.1 (Sia'-C8), 70.6 (Gal'-C3), 71.2, 71.3 (Gal-C2, Gal'-C5), 72.1, 72.3 (3C, Gal-C5, Sia-C6, Sia'-C6), 72.5 (Gal'-C2), 79.1 (Gal-C3), 96.9, 98.1 (Sia-C2, Sia'-C2), 101.0 (Gal'-C1), 102.9 (Gal-C1), 128.9, 131.0, 133.2 (C_6H_5), 165.7, 167.7, 168.0, 170.0, 170.2, 170.3, 170.4, 170.4, 170.5, 170.6, 170.7, 170.7, 170.8, 171.0, 171.4, 172.3 (16 CO); HRMS (FAB) Calcd for $C_{70}H_{98}N_2NaO_{39}Si$ $[M+Na]^+$: 1641.5414; Found: 1641.5434.

2-(Trimethylsilyl)ethyl (sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-(β-D-galactopyranosyl)-(1→3)-[(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)]-(2→6)-β-D-galactopyranoside (3). A solution of **20**a (30 mg, 0.02 mmol) in MeOH (2.5 mL) was treated with freshly prepared 1 M NaOMe/MeOH (0.3 mL) for 7 h at r.t. under argon. Then water (1 mL) was added and the mixture stirred for another 16 h at r.t. The solution was concentrated and the residue purified by reversed-phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **3** (9.6 mg, 49%) as a colorless solid after a final lyophilization from water.

$[\alpha]_D$ +1.5 (*c* 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.00 (s, 9H, $SiMe_3$), 0.95 (dt, *J* = 5.2, 12.9 Hz, 1H, C*H*$_2$Si-Ha), 1.04 (dt, *J* = 5.5, 12.9 Hz, 1H, C*H*$_2$Si-Hb), 1.63 (t, *J* = 12.1 Hz, 1H, Sia'-H3a), 1.77 (t, *J* = 12.1 Hz, 1H, Sia-H3a), 2.00 (s, 6H, 2 $COCH_3$), 2.68 (dd, *J* = 4.6, 12.4 Hz, 1H, Sia'-H3b), 2.73 (dd, *J* = 4.6, 12.4 Hz, 1H, Sia-H3b), 3.58-3.68 (m, 13H, Gal-H2, Gal-H6a, Gal'-H2, Gal'-H6, Sia-H4, Sia-H6, Sia-H7, Sia-H9a, Sia'-H4, Sia'-H6, Sia'-H7, Sia'-H9a), 3.70-3.88 (m, 10H, Gal-H3, Gal-H5, Gal'-H5, Sia-H5, Sia-H8, Sia-H9b, Sia'-H5, Sia'-H8, Sia'-H9b, OC*H*$_2$-Ha), 3.89-3.92 (m, 2H, Gal-H6b, Gal'-H4), 4.00 (ddd, *J* = 5.2, 10.1, 12.7 Hz, 1H, OC*H*$_2$-Hb), 4.08 (dd, *J* = 3.1, 9.8 Hz, 1H, Gal'-H3), 4.18 (d, *J* = 3.4 Hz, 1H, Gal-H4), 4.42 (d, *J* = 8.0 Hz, 1H, Gal-H1), 4.63 (d, *J* = 7.8 Hz, 1H, Gal'-H1); ^{13}C NMR (125 MHz, D_2O): δ -2.6 ($SiMe_3$), 17.5 (CH_2Si), 22.0, 22.0 (2 CO*C*H_3), 39.6, 40.3 (Sia-C3, Sia'-C3), 51.6, 51.8 (Sia-C5, Sia'-C5), 60.9 (Gal'-C6), 62.5 (Sia'-C9), 62.5 (Sia-C9), 63.1 (Gal-C6), 67.4 (Gal'-C4), 68.0, 68.1, 68.3 (Sia-C4, Sia-C7, Sia'-C4), 68.3 (Gal-C4), 68.4 (OCH_2), 69.4, 69.6 (3C, Gal-C5, Gal'-C5, Sia'-C7), 71.6, 71.7 (Sia-C6, Sia'-C6), 72.6, 72.8 (Sia-C8, Sia'-C8), 74.8 (2C, Gal-C2, Gal'-C2), 75.5 (Gal'-C3), 82.6 (Gal-C3), 99.7, 100.3 (Sia-C2, Sia'-C2), 101.7 (Gal-C1), 104.1 (Gal'-C1), 173.4, 173.8, 174.9, 175.0 (4 CO); HRMS (FAB) Calcd for $C_{39}H_{66}N_2O_{27}Si$ $[M-2H]^{2-}$: 511.1811; Found: 511.1817.

3,4,6-Tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hexitol (22). To a solution of 3,4,6-tri-*O*-acetyl-D-galactal **21** (6.29 g, 23.1 mmol) in MeOH (7 mL) was added palladium on charcoal (10%, 1.00 g) and the suspension was stirred at r.t. for 3 h under an atmosphere of hydrogen (1 bar). The mixture was filtered through a pad of Celite, the Celite was washed with MeOH (2 × 5 mL) and the combined filtrates were concentrated under reduced pressure. The residue was purified by silica gel chromatography (petrol ether/EtOAc 2:1) to afford **22** (6.16 g, 97%) as a colorless oil.

$[\alpha]_D$ +51.7 (*c* 1.01, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 1.71 (m, 1H, H-2a), 1.99, 2.05 (2s, 6H, 2 $COCH_3$), 2.09 (m, 1H, H-2b), 2.13 (s, 3H, $COCH_3$), 3.37 (dt, *J* = 2.2, 12.4 Hz, 1H, H-1a), 3.74 (m, 1H, H-5), 4.07-4.10 (m, 2H, H-6), 4.11 (m, 1H, H-1b), 4.98 (dt, *J* = 2.6, 12.1 Hz, 1H, H-3), 5.29 (m, 1H, 4-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7, 20.7, 20.8 (3 $COCH_3$), 26.3 (C-2), 62.7 (C-6), 65.8 (C-1), 68.8 (C-4), 69.6 (C-3), 74.8 (C-5), 170.1, 170.3, 170.6 (3 CO); ESI-MS Calcd for $C_{12}H_{18}NaO_7$ $[M+Na]^+$: 297.10; Found: 297.13.

1,5-Anhydro-2-deoxy-D-*lyxo*-hexitol (23). A solution of **22** (6.16 g, 22.5 mmol) in MeOH (5 mL) was treated with 1 M methanolic NaOMe (0.5 mL) under argon at r.t. for 3 h. The reaction mixture was neutralized with Dowex 50X8 (H^+ type) ion-exchange resin and filtered through a pad of Celite. The Celite was washed with methanol (3 × 5 mL), and the combined filtrates were evaporated to dryness to give pure **23** (3.30 g, 99%) as a colorless solid.
$[\alpha]_D$ +36.8 (*c* 1.05, MeOH); ^1H NMR (500 MHz, D_2O): δ 1.69 (m, 1H, H-2a), 1.83 (m, 1H, H-2b), 3.46-3.52 (m, 2H, H-1a, H-5), 3.66 (dd, *J* = 4.2, 11.7 Hz, 1H, H-6a), 3.71 (dd, *J* = 8.0, 11.7 Hz, 1H, H-6b), 3.81 (m, 1H, 4-H), 3.83 (m, 1H, H-3), 3.99 (m, 1H, H-1b); ^{13}C NMR (125 MHz, D_2O): 28.1 (C-2), 62.3 (C-6), 66.0 (C-1), 68.3 (C-4), 69.1 (C-3), 79.4 (C-5); ESI-MS Calcd for $C_6H_{12}NaO_4$ $[M+Na]^+$: 171.06; Found: 171.10.

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-D-*lyxo*-hexitol (24). To a solution of **23** (2.20 g, 14.9 mmol) and benzaldehyde dimethyl acetal (3.34 mL, 22.7 mmol) in MeCN (110 mL) was added *p*-toluene sulfonic acid monohydrate (801 mg, 4.21 mmol). The mixture was stirred at r.t. for 18 h before NEt_3 (1 mL) was added. The solvent was removed, and the residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 2:1) to yield **24** (2.96 g, 84%) as a colorless solid.
$[\alpha]_D$ +45.5 (*c* 1.02, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 1.78 (m, 1H, H-2a), 2.01 (m, 1H, H-2b), 2.36 (d, *J* = 10.9, 1H, OH), 3.33 (m, 1H, H-5), 3.48 (m, 1H, H-1a), 3.78 (m, 1H, H-3), 4.04 (A of AB, *J* = 12.4 Hz, 1H, H-6A), 4.11 (m, 1H, 4-H), 4.13 (m, 1H, H-1b), 4.29 (B of AB, *J* = 12.4 Hz, 1H, H-6B), 5.60 (s, 1H, C*H*Ph), 7.36-7.42, 7.51-7.53 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): 30.2 (C-2), 65.8 (C-1), 68.8 (C-3), 70.1 (C-5), 70.3 (C-6), 75.2 (C-4), 101.2 (*C*HPh), 126.3, 128.2, 129.1, 137.7 (6C, C_6H_5); ESI-MS Calcd for $C_{13}H_{16}NaO_4$ $[M+Na]^+$: 259.10; Found: 259.09.

(Benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-1,5-an-

hydro-4,6-*O*-benzylidene-2-deoxy-D-*lyxo*-hexitol (25). A mixture of **24** (70.9 mg, 0.300 mmol), **7**[S22] (145 mg, 0.150 mmol) and activated powdered molecular sieves 3Å (1.0 g) in DCM (10 mL) was stirred for 2 h at 0°C under argon. Then DMTST (176 mg, 0.600 mmol) was added in one portion. The reaction mixture was stirred at 0°C for additional 2 d, diluted with DCM (10 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3 × 5 mL) and the combined filtrates were washed with saturated aqueous $KHCO_3$ (5 mL) and H_2O (5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH 25:1 to 20:1) to afford **25** (134 mg, 79%) as a colorless foam.

$[\alpha]_D$ +18.2 (*c* 0.50, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.71 (t, *J* = 12.4 Hz, 1H, Sia-H3a), 1.78 (m, 1H, Hex-H2a), 1.82, 1.98, 2.02, 2.06, 2.10, 2.11, 2.14 (7s, 21H, 7 $COCH_3$), 2.18 (m, 1H, Hex-H2b), 2.62 (dd, *J* = 4.6, 12.7 Hz, 1H, Sia-H3b), 3.22 (m, 1H, Hex-H5), 3.48 (m, 1H, Hex-H1a), 3.49 (dd, *J* = 2.7, 10.7 Hz, 1H, Sia-H6), 3.90-3.92 (m, 2H, Hex-H3, Hex-H6a), 3.98 (dd, *J* = 5.5, 12.5 Hz, 1H, Sia-H9a), 4.00-4.05 (m, 2H, Gal-H5, Sia-H5), 4.16 (m, 1H, Hex-H1b), 4.19-4.23 (m, 3H, Hex-H4, Hex-H6b, Gal-H6a), 4.32 (dd, *J* = 2.8, 12.5 Hz, 1H, Sia-H9b), 4.51 (dd, *J* = 6.9, 11.2 Hz, 1H, Gal-H6b), 4.63 (dd, *J* = 3.4, 10.2 Hz, 1H, Gal-H3), 4.83 (d, *J* = 7.9 Hz, 1H, Gal-H1), 4.84 (m, 1H, Sia-H4), 4.91 (d, *J* = 10.3 Hz, 1H, NH), 4.99 (A of AB, *J* = 12.0 Hz, 1H, PhCH_2), 5.13 (dd, *J* = 8.0, 10.1 Hz, 1H, Gal-H2), 5.16 (m, 1H, Gal-H4), 5.31 (m, 1H, Sia-H7), 5.34 (B of AB, *J* = 12.0 Hz, 1H, PhCH_2), 5.52 (m, 1H, Sia-H8), 5.54 (s, 1H, PhC*H*), 7.30-7.38, 7.43-7.46, 7.52-7.58, 8.07-8.08 (m, 15H, 3 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 20.6, 21.3, 22.9 (7C, 7 COCH_3), 29.5 (Hex-C2), 37.2 (Sia-C3), 48.6 (Sia-C5), 61.9 (Gal-C6), 62.3 (Sia-C9), 65.5 (Hex-C1), 66.9 (Sia-C7), 67.5 (Gal-C4), 67.6 (Sia-C8), 68.2 (PhCH_2), 69.1 (Sia-C4), 69.5 (Gal-C2), 70.0 (Hex-C6), 70.1 (Hex-C5), 70.5 (Gal-C5), 71.3 (Gal-C3), 71.8 (Sia-C6), 74.6 (Hex-C4), 75.8 (Hex-C3), 96.6 (Sia-C2), 98.9 (Gal-C1), 100.2 (Ph*C*H), 126.1, 127.8, 128.3, 128.4, 128.5, 128.6, 129.5, 129.6, 133.2, 134.6, 137.9 (18C, 3 C_6H_5), 165.6, 167.3, 169.4, 169.5, 170.1, 170.2, 170.3, 170.4, 170.5 (9 CO); ESI-MS Calcd for $C_{56}H_{65}NNaO_{24}$ $[M+Na]^+$: 1158.38; Found: 1158.43.

(Benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-1,5-an-hydro-2-deoxy-D-*lyxo*-hexitol (26). Compound **25** (125 mg, 0.110 mmol) was dissolved in 80% aqueous acetic acid (4 mL) and stirred for 3 h at 60°C. The solvent was removed in vacuo and the residue co-evaporated with toluene (2 × 5 mL). The remaining solid was

purified by column chromatography on silica gel (DCM/MeOH, 20:1 to 9:1) to give **26** (102 mg, 89%) as a colorless solid.

$[\alpha]_D$ +16.3 (*c* 0.52, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.69 (m, 1H, Hex-H2a), 1.70 (t, *J* = 12.4 Hz, 1H, Sia-H3a), 1.83, 1.99, 2.02 (3s, 9H, 3 $COCH_3$), 2.06 (m, 1H, Hex-H2b), 2.07, 2.12, 2.16, 2.21 (4s, 12H, 4 $COCH_3$), 2.22 (m, 1H, OH), 2.62 (dd, *J* = 4.6, 12.6 Hz, 1H, Sia-H3b), 2.67 (m, 1H, OH), 3.30 (m, 1H, Hex-H5), 3.44 (m, 1H, Hex-H1a), 3.49 (dd, *J* = 2.7, 10.8 Hz, 1H, Sia-H6), 3.54 (m, 1H, Hex-H6a), 3.78 (m, 1H, Hex-H3), 3.82 (m, 1H, Hex-H6b), 3.95 (m, 1H, Hex-H4), 3.96 (dd, *J* = 6.0, 12.2 Hz, 1H, Sia-H9a), 4.02-4.10 (m, 3H, Hex-H1b, Gal-H5, Sia-H5), 4.31 (dd, *J* = 5.5, 11.4 Hz, 1H, Gal-H6a), 4.32 (dd, *J* = 2.7, 12.3 Hz, 1H, Sia-H9b), 4.39 (dd, *J* = 7.6, 11.4 Hz, 1H, Gal-H6b), 4.63 (dd, *J* = 3.4, 10.2 Hz, 1H, Gal-H3), 4.77 (d, *J* = 8.0 Hz, 1H, Gal-H1), 4.85 (m, 1H, Sia-H4), 4.95 (d, *J* = 10.3 Hz, 1H, NH), 5.01 (A of AB, *J* = 12.0 Hz, 1H, PhCH_2), 5.07 (dd, *J* = 8.0, 10.2 Hz, 1H, Gal-H2), 5.13 (d, *J* = 3.0 Hz, 1H, Gal-H4), 5.31 (dd, *J* = 2.7, 9.3 Hz, 1H, Sia-H7), 5.38 (B of AB, *J* = 12.0 Hz, 1H, PhCH_2), 5.56 (ddd, *J* = 2.7, 5.8, 9.3 Hz, 1H, Sia-H8), 7.36-7.48, 7.57-7.60, 8.07-8.08 (m, 10H, 2 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 20.6, 20.7, 20.7, 20.7, 21.4, 22.9 (7 COCH_3), 26.2 (Hex-C2), 37.3 (Sia-C3), 48.6 (Sia-C5), 62.0 (Gal-C6), 62.4 (Sia-C9), 63.3 (Hex-C6), 65.6 (Hex-C1), 66.9 (Sia-C7), 67.5, 67.5 (Gal-C4, Sia-C8), 68.0 (Hex-C4), 68.3 (PhCH_2), 69.1 (Sia-C4), 69.5 (Gal-C2), 70.8 (Gal-C5), 71.0 (Gal-C3), 71.8 (Sia-C6), 78.0 (Hex-C5), 78.5 (Hex-C3), 96.6 (Sia-C2), 99.9 (Gal-C1), 128.4, 128.5, 128.6, 128.8, 129.4, 129.6, 133.4, 134.6 (12C, 2 C_6H_5), 165.8, 167.3, 169.6, 169.7, 170.1, 170.4, 170.4, 170.5, 170.7 (9 CO); ESI-MS Calcd for $C_{49}H_{61}NNaO_{24}$ $[M+Na]^+$: 1070.35; Found: 1070.44.

(Benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α**-D-*galacto*-2-nonulo-pyranosynate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzoyl-**β**-D-galactopyranosyl)-(1→3)-1,5-anhydro-2-deoxy-6-*O*-[(*S*)-1-ethoxycarbonyl-ethyl]-D-*lyxo*-hexitol (28).** A suspension of compound **26** (50.0 mg, 47.7 μmol) and Bu_2SnO (12.5 mg, 50.1 μmol) in benzene (2 mL) was stirred at 80°C under argon for 3 h. After complete removal of the solvent in vacuo, the residue was redissolved in DME (2 mL). Ethyl (*R*)-2-*O*-(trifluoromethylsulfonyl)-lactate **27**[S25] (26.7 μL, 143 μmol) and freshly dried CsF (29.1 mg, 191 μmol) were subsequently added and the reaction was stirred at r.t. under argon for 20 h. The mixture was diluted with DCM (10 mL), washed with saturated aqueous $NaHCO_3$ (5 mL) and water (5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH, 20:1 to 10:1) to yield **28** (45.6 mg, 83%) as a colorless solid.

$[\alpha]_D$ +2.5 (*c* 0.51, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.26 (m, 3H, OCH_2CH_3), 1.39 (d, *J* = 6.9 Hz, 3H, Lac-H3), 1.69 (t, *J* = 12.4 Hz, 1H, Sia-H3a), 1.70 (m, 1H, Hex-H2a), 1.82, 1.97, 2.01 (3s, 9H, 3 $COCH_3$), 2.03 (m, 1H, Hex-H2b), 2.06, 2.10, 2.14, 2.19 (4s, 12H, 4 $COCH_3$), 2.61 (dd, *J* = 4.5, 12.6 Hz, 1H, Sia-H3b), 3.42 (m, 1H, Hex-H1a), 3.48-3.51 (m, 2H, Hex-H5, Sia-H6"), 3.60 (dd, *J* = 4.0, 10.3 Hz, 1H, Hex-H6a), 3.63 (dd, *J* = 7.5, 10.3 Hz, 1H, Hex-H6b), 3.79 (m, 1H, Hex-H3), 3.94 (m, 1H, Hex-H4), 3.95-4.07 (m, 5H, Hex-H1b, Gal-H5, Sia-H5, Sia-H9a, Lac-H2), 4.12-4.22 (m, 2H, OCH_2CH_3), 4.26 (dd, *J* = 6.4, 11.2 Hz, 1H, Gal-H6a), 4.32 (dd, *J* = 2.6, 12.4 Hz, 1H, Sia-H9b), 4.40 (dd, *J* = 7.1, 11.2 Hz, 1H, Gal-H6b), 4.62 (dd, *J* = 3.4, 10.2 Hz, 1H, Gal-H3), 4.78 (d, *J* = 8.0 Hz, 1H, Gal-H1), 4.83 (m, 1H, Sia-H4), 4.97 (A of AB, *J* = 12.0 Hz, 1H, $PhCH_2$), 4.99 (d, *J* = 9.8 Hz, 1H, NH), 5.06 (dd, *J* = 8.0, 10.1 Hz, 1H, Gal-H2), 5.13 (d, *J* = 3.1 Hz, 1H, Gal-H4), 5.30 (dd, *J* = 2.1, 9.3 Hz, 1H, Sia-H7), 5.33 (B of AB, *J* = 12.0 Hz, 1H, $PhCH_2$), 5.55 (ddd, *J* = 2.6, 5.9, 9.3 Hz, 1H, Sia-H8), 7.33-7.46, 7.52-7.57, 8.05-8.07 (m, 10H, 2 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.2 (OCH_2CH_3), 18.6 (Lac-C3), 20.7, 20.7, 20.8, 20.8, 21.4, 23.1 (7C, 7 $COCH_3$), 26.1 (Hex-C2), 37.3 (Sia-C3), 48.8 (Sia-C5), 60.8 (OCH_2CH_3), 61.8 (Gal-C6), 62.5 (Sia-C9), 65.6 (Hex-C1), 67.0 (Sia-C7), 67.5, 67.5 (3C, Hex-C4, Gal-C4, Sia-C8), 68.4 ($PhCH_2$), 69.2 (Sia-C4), 69.6 (Gal-C2), 70.7 (Gal-C5), 70.8 (Hex-C6), 71.2 (Gal-C3), 71.9 (Sia-C6), 75.3 (Lac-C2), 77.6 (Hex-C5), 78.1 (Hex-C3), 96.7 (Sia-C2), 99.6 (Gal-C1), 128.4, 128.6, 128.6, 128.8, 129.6, 129.7, 133.3, 134.6 (12C, 2 C_6H_5), 165.8, 167.4, 169.6, 169.7, 170.1, 170.3, 170.4, 170.5, 170.6, 173.3 (10 CO); ESI-MS Calcd for $C_{54}H_{69}NNaO_{26}$ $[M+Na]^+$: 1170.40; Found: 1170.49.

(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-β-D-galactopyranosyl-(1→3)-1,5-anhydro-2-deoxy-6-*O*-[sodium (*S*)-1-carboxylato-ethyl]-D-*lyxo*-hexitol (4). A solution of **39** (42.0 mg, 36.6 μmol) in MeOH (3 mL) was treated with 1 M NaOMe/MeOH (0.2 mL) for 24 h. Then water (0.5 mL) was added and the mixture stirred for another 7 h. The solution was concentrated and the residue purified by reversed-phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **4** (21.3 mg, 81%) as a colorless solid after a final lyophilization from water.

$[\alpha]_D$ +3.0 (*c* 0.71, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.30 (d, *J* = 6.8 Hz, 1H, Lac-H3), 1.79 (t, *J* = 12.1 Hz, 1H, Sia-H3a), 1.89 (m, 2H, Hex-H2), 2.02 (s, 3H, $COCH_3$), 2.75 (dd, *J* = 4.6, 12.4 Hz, 1H, Sia-H3b), 3.49-3.54 (m, 2H, Hex-H1a, Hex-H6a), 3.56 (dd, *J* = 7.9, 9.7 Hz, 1H, Gal-H2), 3.57-3.71 (m, 8H, Hex-H5, Hex-H6b, Gal-H5, Gal-H6a, Sia-H4, Sia-H6, Sia-H7, Sia-H9a), 3.74 (dd, *J* = 7.9, 11.7 Hz, 1H, Gal-H6b), 3.82-3.89 (m, 4H, Sia-H5, Sia-H8, Sia-

H9b, Lac-H2), 3.94 (d, *J* = 3.0 Hz, 1H, Gal-H4), 4.01-4.05 (m, 3H, Hex-H1b, Hex-H3, Hex-H4), 4.09 (dd, *J* = 3.1, 9.8 Hz, 1H, Gal-H3), 4.62 (d, *J* = 7.9 Hz, 1H, Gal-H1); ^{13}C NMR (125 MHz, D_2O): δ 18.7 (Lac-C3), 22.4 ($COCH_3$), 25.8 (Hex-C2), 40.0 (Sia-C3), 52.1 (Sia-C5), 61.3 (Gal-C6), 62.9 (Sia-C9), 66.0 (Hex-C1), 67.9 (Gal-C4), 68.2 (Hex-C4), 68.5 (Sia-C7), 68.8 (Sia-C4), 69.5 (Gal-C2), 70.2 (Hex-C6), 72.1 (Sia-C8), 73.2 (Sia-C6), 75.2 (Gal-C5), 76.0 (Gal-C3), 77.2 (Hex-C3), 77.8 (Hex-C5), 78.3 (Lac-C2), 100.2 (Sia-C2), 101.6 (Gal-C1), 174.3, 175.4 (3C, 3 CO); HRMS (ESI-FT-ICR) Calcd for $C_{26}H_{43}NO_{19}$ $[M-H]^-$: 672.2357; Found: 672.2388.

In vitro binding assay:

For the inhibition assays and the NMR experiments, a recombinant protein consisting of the N-terminal three domains of MAG and the Fc part of human IgG (Fc-MAG_{d1-3}) was produced by expression in CHO cells and affinity purification on protein A-agarose as described.[S26]

For the hapten inhibition assays a modification of the previously described assay[S27] was used. Instead of human erythrocytes as target for sialic acid-dependent binding of MAG, commercially available microtiter plates with immobilized Neu5Ac (Lundonia, Lund, Sweden) were used. In brief, to each well 10 µl of an oligosaccharide solution was added followed by 20 µl of Fc-MAG which had been precomplexed with an anti-Fc antibody labelled with alkaline phosphatase. After an overnight incubation at 4°C unbound Fc-MAG complexes were removed by washing and the amount of bound Fc-MAG was determined via the alkaline phosphatase with fluorescein diphosphate as substrate. For each oligosaccharide at least eight concentrations were used to determine the concentration required for 50% inhibition (IC_{50}). In order to compare the results from different assays compound **5** was included in each test and used as a reference to calculate the relative inhibitory potencies (rIP). At least three independent titrations were performed for each compound.

NMR experiments

All NMR spectra were recorded on a BRUKER DRX 500 spectrometer. Data acquisition and processing were performed with XWINNMR software (BRUKER) running on Silicon Graphics Indy and O2 workstations. The measurements were performed at 288 K using –$O(CH_2)_2SiMe_3$ (-OSE) as internal reference (0 ppm) for all the experiments except tetrasaccharide **3** which was measured at 280 K. The resulting trNOEs were significant and negative. trNOEs and NOEs were integrated with the program XWINNMR (BRUKER). Phase sensitive NOESY experiments were performed using States-TPPI with presaturation of

the HDO signal. For all 2D-NOESY spectra, a π/2-shifted squared sine bell-window function was applied in both dimensions prior to the Fourier transformation. After zero filling in t_1, 4K (F_2) × 512 (F_1) data matrices were obtained. For trisaccharide **5** and tetrasaccharide **2**, eight mixing times 50, 75, 100, 150, 250, 350, 500 and 750 ms were chosen to generate the NOE build-up curves whereas 500 ms mixing time was used for tetrasaccharide **3**, pseudo-tetrasaccharide **4** and trisaccharide **6**. Unless otherwise stated 2D trNOESY spectra were recorded with 256 increments in t1 and 4K data points in t2. The spectral width was normally 11 ppm in both dimensions. A spin lock pulse with strength of 18 dB and duration of 15 ms was applied after the first $\frac{\pi}{2}$ pulse to suppress protein ^{1}H NMR signals. After 16 dummy scans, 64 scans were recorded per t1 increment. The residual HDO signal was presaturated with a weak rf field (74-76 dB) during relaxation and mixing time. A gradient pulse (1 ms) at the end of the mixing time was applied to remove the transverse magnetization. Six mixing times 50, 75, 100, 200, 300 and 500 ms were chosen to generate trNOE build-up curves. Each experiment was performed with approximate 18 h measurement time. After zero filling in t1, 4K (t2) × 1K (t1) data matrices were obtained. For all 2D trNOESY spectra, a π/2 shifted squared sine bell-window function was applied in both dimensions prior to the Fourier transformation. To remove Hartman-Hahn artifacts, trROESY experiments were performed with a phase-alternated 180° pulse. The mixing times in the trROESY experiments were varied from 150-350 ms. A relaxation delay of 1.5 s and trROESY spin lock field of 20 dB was applied. The residual HDO signal was presaturated with a weak rf field (74-76 dB) during relaxation and mixing time. Experimental NOE/trNOE build-up curves were fitted to the double-exponential function $f(t) = p0(e^{-p2t})(1-e^{-p1t})$, with p0, p1 and p2 being adjustable parameters. For the calculation of absolute NOEs/trNOEs, the decay curves of the diagonal signals were fitted to an exponential function with the form $f(\tau_m) = C \times \exp(-\tau_m / T_{1sel})$, where C is the scaling factor, τ_m is the mixing time and T_{1sel} is the selective spin-lattice relaxation time for extrapolating to a mixing time τ_m of 0 ms. The volume of the diagonal signal at mixing time zero is defined as 100 %, and absolute NOEs are given in percentages.

CORCEMA Calculations

Theoretical transferred NOEs were obtained using a complete relaxation and conformational exchange matrix (CORCEMA) program.[S5,S6] For the CORCEMA calculations a two-state equilibrium involving a ligand and a protein forming a ligand-protein complex was assumed. The program requires (1) the co-ordinates (in pdb format) of the oligosaccharides **3**, **4** and **5** in its free and bound forms, (2) overall rotational correlation times of the complex and (3) the

exchange rates, i.e., off- (k_{off}) and on-rates (k_{on}). As the structure of the complexes of saccharides **2** to **5** with MAG are not known, the protein protons were not evaluated. The following amino acids were used for CORCEMA calculation as they were part of the binding pocket: Trp 22, Tyr 60, Ser 63-Tyr 69 and Arg 118-Ser 130. For the calculations, an overall isotropic motion of the complex was assumed, and internal motion was neglected. The calculations were performed using the K_D values obtained from the STD titration curves.[S28] A grid search was performed in which the off-rate was incremented from 10 to 50 s^{-1} by 5 steps. These calculations were performed at different overall correlation times varying from 15 to 55 ns by 5 ns steps in the bound state. Finally, an overall correlation time of 40 ns was found to correspond well within the experimental data. For the free oligosaccharides **3**, **4** and **5**, a correlation time of 0.3, 0.5 and 0.35 ns, respectively, yielded a good approximation of experimental NOEs. The program calculates the cross-peak intensities separately for the direct NOESY cross-peaks and for the exchange-mediated cross-peaks. For the comparison with experimental data these contributions were added. From the comparison of theoretical trNOEs with experimental trNOEs, R-factors were calculated (eq. 1):

$$R-\text{factor}=\sqrt{\frac{\sum(|NOE_{exp}-NOE_{cal}|)^2}{\sum(|NOE_{exp}|)^2}} \qquad (1)$$

NOE_{exp} and NOE_{cal} denote experimental and calculated NOEs, respectively. After matching of experimental and theoretical intra-glycosidic trNOE curves, CORCEMA calculations for the inter-glycosidic trNOEs of the ligands were performed.

Homology modeling

On the basis of the primary structure, the extracellular domain of MAG is predicted to be composed of five separate Ig domains. At the amino acid sequence level, the first two Ig domains of MAG share a significant degree of homology with other Ig family members, such as sialoadhesin.[S29] The first extracellular domain of all the family members is a V-type Ig domain, containing the Neu5Ac binding site. The structure of this domain from sialoadhesin has recently been determined by X-ray crystallography in complex with the ligand α(2→3)-sialyllactose.[S1] This crystal structure was used as a template for homology modeling of the binding domain (N-terminal V-set domain) of MAG (Swiss-Prot ID P20917). Homology modeling was conveniently implemented in the COMPOSER option of the Sybyl software

suite (TRIPOS Associates, USA). The program performed a pairwise sequence alignment. Loops missing in the sialoadhesin structure were subsequently generated. Finally, an energy minimization was performed with the Tripos force field (TRIPOS Associates, USA). The refined model was checked for the stereochemical parameters by the program PROCHECK.[S2]

AutoDock 3.0

Computational docking simulations were performed with AutoDock 3.0.[S3] Partial conformations of ligands (tetrasaccharide **3** and pseudo-tetrasaccharide **4**) were extracted from the crystal structure of α(2→3)-sialyllactose (acquisition code, 1QFO). The protein models were used by Kollmann all atom charges using SYBYL 6.9/Biopolymer. Grid maps representing the proteins were constructed using 127 × 127 × 127 points, with grid spacing of 0.375 Å, and centered on the ligand, which was manually positioned within the binding site. No torsions of the ligand were allowed to vary during the docking. Protein-ligand complexes were generated from this starting point using a Lamarckian genetic algorithm (LGA).[S3] Parameters for LGA docking were used as recommended in the AutoDock manual. For each protein-ligand pair, 100 LGA docking runs were performed. The maximum number of accepted and rejected trials per cycle was set to 10^6. The binding modes were clustered using a RMS deviation cutoff of 1.0 Å with respect to the starting position. The inter-molecular energy of the lowest energy cluster was used for all plots in this study. Inter-molecular energy refers to the potential energy of the interaction between the protein and ligand (van der Waals, electrostatic, hydrogen bonding and desolvation free energy components); it does not include the internal energies of the protein and ligand themselves.

References

[S1] A. P. May, R. C. Robinson, M. Vinson, P. R. Crocker, E. Y. Jones, *Mol. Cell* **1998**, *1*, 719-728.

[S2] R. A. Laskowski, J. A. Rullmannn, M. W. MacArthur, R. Kaptein, J. M. Thornton, *J. Biomol. NMR* **1996**, *8*, 477-486.

[S3] G. A. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comp. Chem.* **1998**, *19*, 1639-1662.

[S4] L. Poppe, R. Stuike-Prill, B. Meyer, H. van Halbeek, *J. Biomol. NMR* **1992**, *2*, 109-136.

[S5] E. V. Curto, H. N. Moseley, N. R. Krishna, *J. Comput. Aided Mol. Des.* **1996**, *10*, 361-371.

[S6] H. N. Moseley, E. V. Curto, N. R. Krishna, *J. Magn. Reson. B* **1995**, *108*, 243-261.

[S7] A. A. Vyas, O. Blixt, J. C. Paulson, R. L. Schnaar, *J. Biol. Chem.* **2005**, *280*, 16305-16310.

[S8] S. Tang, Y. J. Shen, M. E. DeBellard, G. Mukhopadhyay, J. L. Salzer, P. R. Crocker, M. T. Filbin, *J. Cell Biol.* **1997**, *138*, 1355-1366.

[S9] A. Imberty, C. Gautier, J. Lescar, S. Pérez, L. Wyns, R. Loris, *J. Biol. Chem.* **2000**, *275*, 17541-17548.

[S10] S. Swaminathan, S. Eswaramoorthy, *Acta Cryst.* **2000**, *D56*, 1024-1026.

[S11] C. Fotinou, P. Emsley, I. Black, H. Ando, H. Ishida, M. Kiso, K. A. Sinha, N. F. Fairweather, N. W. Isaacs, *J. Biol. Chem.* **2001**, *276*, 32274-32281.

[S12] W. S. Somers, J. Tang, G. D. Shaw, R. T. Camphausen, *Cell* **2000**, *103*, 467-479.

[S13] Y. Ha, D. J. Stevens, J. J. Skehel, D. C. Wiley, *Proc. Natl. Acad. Sci.* **2001**, *98*, 11181-11186.

[S14] T. Stehle, S. C. Harrison, *Structure* **1996**, *4*, 183-194.

[S15] K. K.-S. Ng, W. I. Weis, *Biochemistry* **1997**, *36*, 979-988.

[S16] E. A. Merritt, S. Sarfaty, M. G. Jobling, T. Chang, R. K. Holmes, T. R. Hirst, W. G. Hol, *Protein Sci.* **1997**, *6*, 1516-1528.

[S17] E. A. Merritt, P. Kuhn, S. Sarfaty, J. L. Erbe, R. K. Holmes, W. G. J. Hol, *J. Mol. Biol.* **1998**, *282*, 1043-1059.

[S18] K. Scheffler, B. Ernst, A. Katopodis, J. L. Magnani, W. T. Wang, R. Weisemann, T. Peters, *Angew. Chem.* **1995**, *107*, 2034-2037, *Angew. Chem. Int. Ed.* **1995**, *34*, 1841-1844.

[S19] L. Poppe, G. S. Brown, J. S. Philo, P. V. Nikrad, B. H. Shah, *J. Am. Chem. Soc.* **1997**, *119*, 1727-1736.

[S20] R. Harris, G. R. Kiddle, R. A. Field, M. J. Milton, B. Ernst, J. L. Magnani, S. W. Homans, *J. Am. Chem. Soc.* **1999**, *121*, 2546-2551.

[S21] T. Haselhorst, T. Weimar, T. Peters, *J. Am. Chem. Soc.* **2001**, *123*, 10705-10714.

[S22] Donor **7** was synthesized in analogy to the procedure for methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside published by A. Kameyama, H. Ishida, M. Kiso, A. Hasegawa *Carbohydr. Res.* **1988**, *184*, c1-c4; A. Kameyama, H. Ishida, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.* **1989**, *8*, 799-804.

[S23] O. Schwardt, G.-P. Gao, T. Visekruna, S. Rabbani, E. Gassmann, B. Ernst, *J. Carbohydr. Chem.* **2004**, *23*, 1-28.

[S24] O. Kanie, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.* **1988**, *7*, 501-506.

[S25] H. Paulsen, P. Himpkamp, T. Peters, *Liebigs Ann. Chem.* **1986**, 664-674.

[S26] P. R. Crocker, S. Kelm in *Weir's Handbook of Experimental Immunology*, (Eds.: D.M. Herzenberg, L. A. Weir), 5th edition, vol. 4, Blackwell Science, Boston **1996**, pp 166.1-166.11.

[S27] a) S. Kelm, R. Brossmer, R. Isecke, H.-J. Gross, K. Strenge, R. Schauer, *Eur. J. Biochem.* **1998**, *255*, 663-672; b) K. Strenge, R. Schauer, N. Bovin, A. Hasegawa, H. Ishida, M. Kiso, S. Kelm, *Eur. J. Biochem.* **1998**, *258*, 677-685.

[S28] S.-Y. Shin, H. Gäthje, O. Schwardt, G.-P. Gao, B. Ernst, S. Kelm, B. Meyer, unpublished results.

[S29] S. Kelm, R. Schauer, P. R. Crocker, *Glycoconj. J.* **1996**, *13*, 913-926.