Synthesis of Antagonists of Myelin-associated Glycoprotein (MAG) for Conformational and SAR Studies

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Dekan

To: Xiao-an

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Declaration

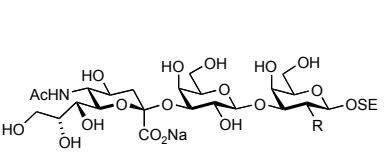
I declare, that I wrote this thesis "Synthesis of antagonists of Myelin-associated glycoprotein (MAG) for conformational and SAR studies" with the help indicated and only handed it in to the faculty of science of the University of Basel and to no other faculty and other university.

Gan-Pan Gao,
Basel, August 2004

Abstract

Damage to the central nervous system (CNS) of higher vertebrates, including humans, often results in devastating and persistent functional deficits. The limited capacity of the adult mammalian CNS to repair lesions by axonal regeneration is mainly caused by the inhibitory molecules in myelin: Myelin-associated glycoprotein (MAG), Nogo, and Oligodendrocyte-myelin glycoprotein (OMgp).

Gangiosides, such as GD1a, GT1b and GQ1bα, are specific functional ligands responsible for MAG-mediated inhibition of neurite outgrowth. Based on the previous SAR studies, partial structure of natural ligands and derivatives thereof (**1**, **20**, **45**, **54α** and **54β**) were chemically and chemo-enzymatically synthesized.



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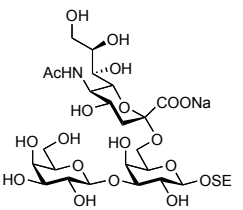
20 R = OH



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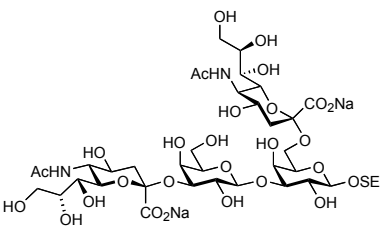

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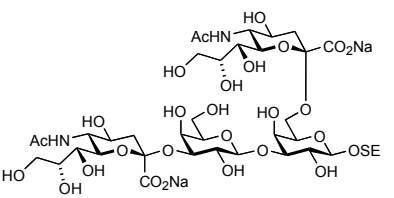
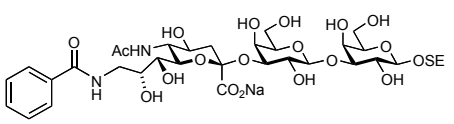


54β

Their biological affinities were tested in a fluorescent hapten inhibition assay; the binding epitopes were identified by STD NMR; and the bioactive conformations were deduced by trNOEs NMR. This led to the design of two families of novel mimics with either modifications at the C-9 position of the α(2,3)-linked terminal sialic acid (**87**, **97**), or substitutions of the disaccharide core by non-carbohydrate moieties (**104**-**107**, **120**-**122**).



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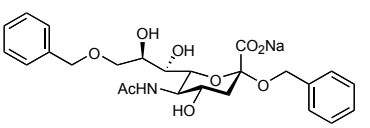

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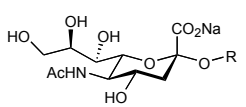
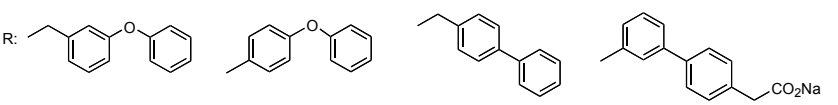
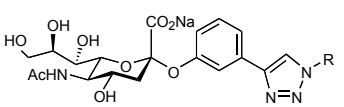
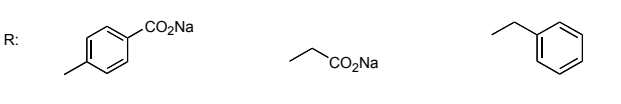




104 **105** **106** **107**





120 **121** **122**

Abbreviations

τ_c	motional correlation or tumbling time
Ac	acetyl
BHT	butylated hydroxytoluene
Bn	benzyl
BSA	bovine serum albumin
Bz	benzoyl
CAM	cell adhesion molecules
CAN	cerium ammonium nitrate
CHO cell	Chinese hamster ovary cell
CIAP	calf intestine alkaline phosphatase
CMP	cytidine monophosphate
CNS	central nervous system
CSA	camphor sulfonic acid
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DEAE anion	diethylaminoethyl anion
DMAP	4-dimethylaminopyridine
DMF	*N*, *N*'-dimethylformamide
DMTST	dimethyl(methylthio)sulfonium triflate
dppf	1,1'-Bis(diphenylphosphino)ferrocene
DRG	dorsal root ganglion
ESI-MS	electrospray ionization mass spectrometry
FDP	fluorescein diphosphate
Fuc	fucose
Gal	galactose
Gal*N*Ac	*N*-acetyl galactosamine
Glc	glucose
GPI	glycosylphosphatidylinositol
HRMS (FAB)	high resolution mass spectrometry (fast-atom bombardment)
IC_{50}	concentration required for 50% inhibition
i-PrOH	2-propanol
K_D	dissociation constance
kDa	kiloDalton
KDN	5-deaminated neuraminic acid
K_i	inhibitory constance
$logD_{7.4}$	*n*-octanol/water partition coefficient at pH 7.4

LRR	leucine rich repeat
MAG	Myelin-associated glycoprotein
MAG-/-	MAG-deficient
MAG+/+	MAG-wild type
MPM	4-methoxy phenylmethyl
Neu5Ac	*N*-acetylneuraminic acid
NeuGc	*N*-glycolyl neuraminic acid
NgR	Nogo receptor
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
OMgp	Oligodendrocyte-myelin glycoprotein
OSE	2-(trimethylsilyl)ethyl
P4	1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol
PE	petrolether
PND	post-natal day
PNS	peripheral nervous system
py	pyridine
R118A	mutation of Arg118 to Ala
R118D	mutation of Arg118 to Asp
RG	retinal ganglion
rIP	relative inhibitory potency
RP	reversed phase
SAR	structure-activity relationship
Sia	sialic acid
siglec	sialic acid binding immunoglobulin-like lectin
sLe^X	sialyl $lewis^X$
SMP	Schwann cell myelin protein
Sn	sialoadhesion
ST	sialyltransferase
STD	saturation transfer difference
TBAB	tetrabutylammonium bromide
TBAHS	tetrabutylammonium hydrosulfate
TBAI	tetrabutylammonium iodide
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid, triflic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
trNOE	transfer nuclear overhauser effect
TsOH	*p*-toluenesulfonic acid

Contents

Introduction

Results and Discussion

Introduction

1. Myelin-associated inhibitors of axonal regeneration in adult mammalian CNS

1.1. Failure of axonal regeneration in adult mammalian CNS

Damage to the central nervous system (CNS) of higher vertebrates, including humans, often results in devastating and persistent functional deficits. Hence, victims of stroke, trauma or neurodegenerative diseases suffer permanently from the losses in, *e.g.* movement, body functions, sensation, and thinking.

In contrast to the peripheral nervous system (PNS) and the embryonic CNS, the capacity of the adult brain and spinal cord to repair lesions by axonal regeneration is extremely limited. Although injured axons can sprout spontaneously, this regeneration attempt is transitory and no significant regrowth occurs over long distances.[1] However, this failure is not due to an intrinsic or irreversible lack of the ability of CNS neurons to regenerate, but rather to the non-permissive nature of the CNS environment. This was demonstrated by studies showing that many types of CNS neurons can extend long axons either by grafting pieces of peripheral nerves onto a lesion site,[2,3] or by isolating neurons and growing them in culture.[4,5]

Several factors may account for the regenerative failure observed in the adult mammalian CNS, including a post-natal decline in available neurotrophic factors and intracellular cyclic nucleotides, the formation of a glial scar, the presence of myelin-associated inhibitors of axonal extension, and possibly the presence of developmental repulsive guidance cues.[6] Among them, inhibitors in myelin and glial scar are the two major obstacles to axonal regeneration after injury.[7]

The scar is formed by astrocytes, which change their morphology to act as a physical barrier to axonal outgrowth and also upregulate several extracellular-matrix-associated inhibitors after injury.[8] However, since the glial scar takes a considerable

time to become fully mature, immediately after injury the main impediments to regeneration are inhibitors in myelin.

In the CNS and PNS, many axons are wrapped concentrically and tightly by a fatty sheath of myelin, which is produced by oligodendrocytes in the CNS and Schwann cell in the PNS.[9] Behaving as an insulator, this multilamellar membrane is essential for rapid nerve conduction, as evidenced by debilitating demyelinating diseases in the CNS and PNS such as multiple sclerosis and Guillain Barré syndrome.[10] The myelin sheath is interrupted at regular intervals by the nodes of Ranvier that are relatively small, regularly spaced, unmyelinated regions of axons. In the axonal membrane at these nodes, voltage-gated sodium channels are highly concentrated, thus allowing for the saltatory propagation of the action potential down the length of myelinated axons.[11] The rapid impulse conduction that results from the focal position of sodium channels along the axon has facilitated the evolution of complex, yet compact, nervous system.

Except its insulating function, myelin in the adult mammalian CNS is also recognized as a major inhibitor for axonal regeneration from a variety of neurons both *in vivo* and *in vitro*.[1,12-15] This inhibitory role was strongly confirmed by a study in which mice were immunized with myelin before a spinal cord injury was inflicted. A considerable axonal regeneration across the lesion was detected compared with the control mice that were immunized with liver tissue.[16] Consistent with this view is the observation that lesioned embryonic spinal cord neurons can regenerate for a period that ends at about the same time as the onset of myelination in the spinal cord.[14]

Why does regeneration capacity differ so dramatically between the PNS and CNS, while axons from both systems are wrapped by myelin sheaths? Actually, myelin in the PNS does inhibit neurite outgrowth. Shen's study showed that myelin prepared from the CNS inhibits neurite outgrowth by 70% while PNS myelin inhibits by about 60%, relative to the control membranes.[17] Nearly all the inhibitory molecules identified so far in the CNS myelin are present in the PNS myelin as well. The permissive environment in the PNS for axonal regeneration is at least partly due to the different behavior of Schwann cells and oligodendrocytes. In the PNS, Schwann cells

and macrophages rapidly clear myelin after injury, a process known as Wallerian degeneration.[8] Regeneration takes place only after myelin debris has been cleared, Schwann cells have reverted into a non-myelinating phenotype and the expression of myelin proteins is downregulated.[7] Unlike Schwann cells, oligodendrocytes continue to express myelin proteins—including inhibitors—after injury and they do not engulf myelin debris. In addition, the immune response triggered by the injury in the CNS is much weaker than that in the PNS, *i.e.* fewer macrophages are recruited to the injury site. As a consequence, the removal of myelin in the CNS is much slower after injury, and takes several weeks or month to complete.[18,19] Therefore, myelin–associated inhibitors exposed from the damaged myelin sheath in the CNS are regarded to be the major obstacles for injured axonal regeneration followed by glial scar formation.

1.2. Myelin-associated inhibitors

Following the discovery that myelin is adverse to axonal outgrowth, at least three myelin specific inhibitors—MAG, Nogo, OMgp—have been identified and characterized to date. Substantial progress towards the elucidation of the precise nature of these inhibitory molecules enhanced the understanding of the signaling mechanism involved in the inhibition of axonal regeneration in the adult mammalian CNS at molecular level.

1.2.1. Myelin-associated glycoprotein

Myelin-associated glycoprotein (MAG, *figure* 1), initially described in 1973,[20] was the first protein in myelin to be characterized as an inhibitor of axonal outgrowth.[21,22] MAG is a 100 kDa glycoprotein which has 8 to 9 potential*N*-linked glycosylation sites and consists of typically 30% carbohydrate by weight.[23] As a member of siglec (*s*ialic acid binding *i*mmuno*g*lobulin-like *lec*tin) family[24], MAG contains five Ig-like domains in its extracellular sequence and the first Ig-domain adopts an unusual conformation by folding over the second Ig-domain. This transmembrane protein exists in two alternatively spliced isoforms, a large (L) and a small (S) form that differ only in their cytoplasmic sequences.[25,26,27] In the CNS, MAG

located in the periaxonal myelin membrane, while in the PNS, it is found in the outermost membrane of the myelin sheath.[28,29]

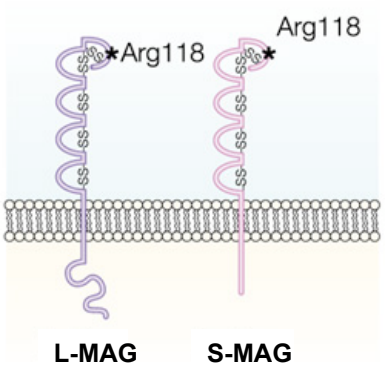


Figure 1: L-MAG and S-MAG.

Because of its localization immediately next to the axon, MAG was suspected to maintain the interface of myelin and axon, and therefore, to influence the integrity of the axon. This idea is greatly strengthened by observations of extensive PNS demyelination and axonal degeneration in MAG-deficient (MAG-/-) mice older than 8 months,[30] while the similar experiment in the CNS has not been reported.

MAG's inhibitory character of axonal regeneration was initially observed by Mckerracher *et al.*[21] They employed octyl glucoside to extract the CNS myelin, followed by chromatography on a diethylaminoethyl (DEAE) anion exchange column, and consistently found MAG as a major component of the multiple neurite growth inhibitory proteins present in the CNS myelin. In addition, immunodepletion of MAG from total extracts of CNS myelin resorted neurite outgrowth up to 63% of control levels, indicating that *in vitro* MAG may contribute significantly to the failure of injured axon to regeneration in the adult mammalian CNS.[21]

Interestingly, depending on age and type, a number of neurons respond to MAG by switching from promotion to inhibition during development.[22,31] For retinal ganglion (RG) neurons and spinal neurons, the switch has already occurred by birth, while for dorsal root ganglion (DRG) neurons, the transition takes place sharply at post-natal day 3 (PND 3). MAG inhibits CNS neurite outgrowth from all post-natal neurons

tested to date, including retinal, superior cervical ganglion, spinal and hippocampal neurons.[31]

To further elucidate the inhibitory role of MAG, MAG-/- mice were created by deletion of the MAG gene by homologus recombination.[32,33] Surprisingly, tests both *in vitro* and *in vivo* showed no significant difference of axonal regeneration between MAG-/- and MAG+/+ mice.[34] The most likely explanation might be that in response to the absence of MAG, other proteins may be upregulated to compensated for MAG, since MAG is just one of the inhibitory factors presented in myelin. Additionally, it is possible that the effect of inhibitory molecules on regeneration is not addictive, therefore the presence of any one inhibitor may be sufficient to prevent most regeneration.

As a member of siglec family, MAG binds to neurons in a sialic-acid dependent manner regardless of whether neurite outgrowth is promoted or inhibited. Importantly, both functions are reduced or even completely abolished, either by desialylation of the neurons by sialidase or by including small sialic-acid-bearing sugars in the cultures.[31]

Arg118 of the first Ig-domain of MAG was recently identified to be crucial for its sialic acid binding capability, because mutation of this amino acid to either Ala (R118A) or Asp (R118D) abolished binding completely. Likewise, inhibition of axonal growth by soluble MAG was lost with this mutation.[35] Surprisingly, when mutated MAG (R118A or R118D) was expressed from either Schwann cell or CHO cells, inhibition was as effective as with native MAG. Therefore, sialic acid binding by itself is not sufficient to effect inhibition of axonal regeneration: there must be a second, yet unknown site on MAG, distinct from the sialic acid binding site at Arg118 for inhibition. However, for soluble MAG, interaction of the inhibition site with the neuron is completely dependent on MAG's inherent sialic acid binding capacity. In contrast, other proteins (CAM, cell adhesion molecules) in live cells can compensate for the MAG's sialic acid binding to the neuron, allowing the inhibition site to interact and to elicit the effort.[35]

1.2.2. Nogo

A first indication that specific molecules in myelin were involved in neurite outgrowth was the IN-1 monoclonal antibody, which was raised against an inhibitory fraction of myelin. In its presence, axons are growing on myelin both *in vitro*[36] and *in vivo*.[1] It was not until a decade later that the antigen for the IN-1 antibody was cloned independently by three groups, which was named Nogo for its inhibitory action on axonal growth (*figure* 2).[37-39] Nogo belongs to the reticulon family, and is expressed as the distinct isoforms A, B, and C in CNS, but not in PNS.[38] Among all three isoforms, Nogo-A is the only one that is expressed in oligodendrocytes, and therefore has been studied extensively. The amino terminus includes at least two inhibitory domains: a specific amino-Nogo that is not shared by Nogo-B and C, and a 66 amino acid loop termed Nogo-66 that is common to all three isoforms. The *C*-terminal region of all three isoforms exhibits a considerable degree of homology (70%) to the reticulon family.[38] Although the topology of Nogo-A has not been clearly established, the model that is best supported by current evidence places Nogo-66 on the extracellular surface, and amino-Nogo on the intracellular surface. As both segments are potent inhibitors of axonal outgrowth, the inhibitory amino terminus may only be exposed following damage to myelin and oligodendrocytes.[7]

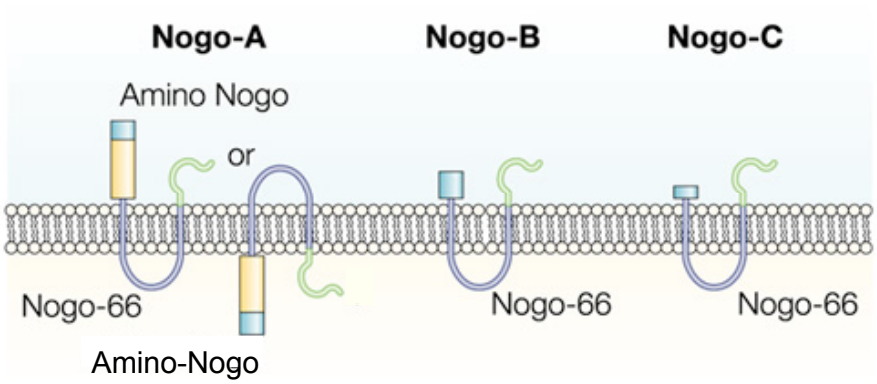


Figure 2: Nogo-A, B, and C.

Recombinant Nogo-A is recognized by the monoclonal antibody IN-1, and it inhibits neurite outgrowth from DRG and spreading of 3T3 fibroblasts in an IN-1 sensitive manner,[37] which showed that Nogo-A is a potent inhibitor of neurite outgrowth.

1.2.3. Oligodendrocyte-myelin glycoprotein

Like MAG, Oligodendrocyte-myelin glycoprotein (OMgp, *figure* 3) was also known long before it was shown to be an inhibitor of axonal regeneration.[40] Except being revealed as the inhibitory component of the fraction of bovine brain myelin by the same strategy as MAG,[41] OMgp was also independently identified as an important myelin-associated inhibitor in a screen for glycosylphosphatidylinositol (GPI)-anchored CNS myelin proteins that mediate axonal outgrowth inhibition.[42] In contrast to its name, OMgp is expressed not only by oligodendrocytes, but also at high levels in various neurons. It is a minor component of myelin with a relative abundance much lower than that of MAG, and is found largely in the paranodal loops, next to the node of Ranvier.[43] OMgp is localized on the outer leaflet of the plasma membrane by a GPI linkage and contains a leucine rich repeat (LRR) domain, followed by a *C*-terminal domain with serine/threonine repeats. *In vitro*, OMgp causes growth cone collapse and potentially inhibits neurite outgrowth, while the function *in vivo* has not yet been reported.[42]

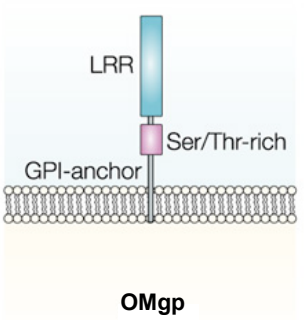


Figure 3: Oligodendrocyte-myelin glycoprotein (OMgp).

1.2.4. Axonal receptors for Myelin-associated inhibitors

A key step in understanding how axons respond to inhibitory influences is to identify the axonal receptors that bind and respond to myelin-associated inhibitory molecules. In 2001, using a soluble form of Nogo-66 to screen a cDNA expression library, Strittmatter cloned a binding partner for Nogo-66 which was termed Nogo receptor (NgR).[44] NgR is an 85 kDa GPI-linked protein that contains eight consecutive LRR domains followed by the *C*-terminal LRR. Direct interaction of Nogo-66 with NgR is required to induce growth cone collapse.[44]

Perhaps one of the most surprising recent findings was the discovery that NgR can also bind and mediate the inhibitory activity of MAG and OMgp.[42,45,46] It is particularly striking since there is no obvious sequence or domain similarity between Nogo-66, MAG and OMgp. A small peptide consisting of the first 40 aa residues of the Nogo-66 sequence, which is essential for binding to NgR but does not contribute to the inhibitory activity, has been found to enhance the majority of axons to regenerate over long distance.[47] In addition, this peptide could also be interfering with the ability of MAG and OMgp to bind to NgR.[7]

NgR is a GPI-linked protein, and therefore has no transmembrane or cytoplasmic domain. It needs a partner to transduce the signal across the membrane. The low-affinity neurotrophin receptor $p75^{NTR}$ was identified as a co-receptor for NgR.[48,49] It can be co-precipitated by MAG, Nogo-66 or OMgp, and NgR is present in the precipitate of each. It was also observed that after injury, upregulation of $p75^{NTR}$ has been shown in many axonal tracts.[50,51] Neurons from $p75^{NTR-/-}$ mice were not inhibited by either of the three inhibitors, or by myelin in general.[7] Furthermore, a truncated $p75^{NTR}$ protein lacking the intracellular domain, when overexpressed in primary neurons, attenuates the same set of inhibitory activities, suggesting that $p75^{NTR}$ is a signal transducer of inhibitory signals into the interior of responding neurons.[48]

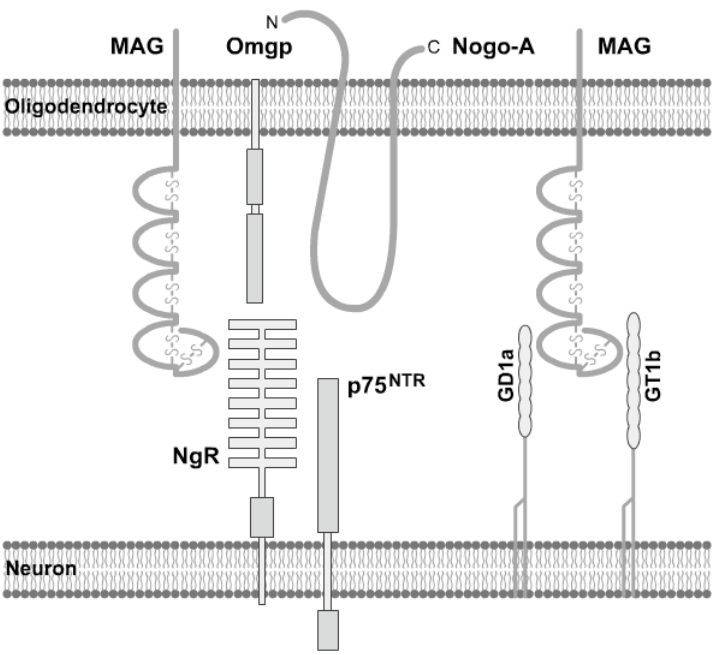


Figure 4: Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS.

MAG, Nogo and OMgp, the three main myelin-associated inhibitors identified to date, interact with the same receptor complex NgR-p75^{NTR} to transduce the inhibitory signal across the membrane, followed by signaling cascades leading to the inhibition of axonal outgrowth after injury (*figure* 4).[52] However, the postulated model implies that there is functional redundancy between these inhibitors. This idea of redundancy can explain many confusing results of *in vivo* experiments in which either MAG or Nogo was blocked or absent. In the MAG-/- mouse, only a small amount of spontaneous axonal regeneration was recorded in one study,[53] and none at all in another.[34] Likewise, application of the antibody to Nogo (IN-1) after injury in wild-type mice allowed improved regeneration to occur, but only 5-10% of axons regrowth.[1,54,55] Presumably, in all of these situations, other ligands for NgR are presented or even upregulated to inhibit axonal regeneration, and the presence of any one inhibitor is sufficient to prevent regeneration by activating an inhibitory signal through a single receptor complex.[8]

The additional inhibitory activity of amino-Nogo which does not act through NgR-p75^{NTR}, and perhaps other as yet unidentified myelin inhibitors might be relatively minor since the blocking of NgR or p75^{NTR} can substantially block the inhibitory effects of total myelin for the neurons tested so far.[7]

1.3. Role of gangliosides in MAG-mediated neurite outgrowth inhibition

Besides the common pathway of MAG, Nogo, and OMgp to transduce the inhibitory signal through the NgR-p75^{NTR} complex, it has long been assumed that MAG mediates neurite outgrowth inhibition by the interaction with gangliosides (*figure* 4). However, the role of gangliosides became debatable after NgR had been identified as the receptor of MAG. In contrast to the opinion that gangliosides can only potentiate and augment MAG-mediated inhibition of neurite outgrowth by facilitating the clustering of signaling molecules,[6] many lines of evidence supports the hypothesis that the nerve cell surface gangliosides are specific functional ligands responsible for MAG-mediated neurite outgrowth inhibition.[10,56,57]

1.3.1. Gangliosides are functional ligands for MAG

Gangliosides, sialic acid-bearing glycosphingolipids, are the major glycans of nerve cells and the major sialic acid-containing glycoconjugates in the brain.[58] They are common to all vertebrate tissues, but 10- to 1000-fold more concentrated in the brain.[59] Brain gangliosides are characterized by their structural diversity, which derives primarily from the number and linkage position of *N*-acetylneuraminic acid (Neu5Ac, sialic acid) residues on the neutral sugar core, whereas the core itself shows only limited varieties. Indicated by a recent study, the major brain gangliosides have the physiological functions to maintain myelin stability, and to control nerve regeneration. Notably, both functions may be mediated, at least in part, *via* their specific interactions with MAG.[10]

Table 1: Established members of the siglec family.[60,61]

Siglec	Alternative name	Tissue/Cell type distribution	Minimal carbohydrate structure(s) recognized
Siglec-1	Sialoadhesin	Macrophages in spleen, lymph nodes, and bone marrow	Neu5Ac-α(2-3)-Gal-β(1-3/4)-Glc*N*Ac- Neu5Ac-α(2-3)-Gal-β(1-3)-Gal*N*Ac-
Siglec-2	CD22	B cells	Neu5Ac-α(2-6)-Gal-β(1-4)-Glc*N*Ac-
Siglec-3	CD33	Myeloid cell lineage	Neu5Ac-α(2-3)-Gal-β(1-3/4)-Glc*N*Ac- Neu5Ac-α(2-3)-Gal-β(1-3)-Gal*N*Ac-
Siglec-4a	MAG	Peripheral and central nerve system	Neu5Ac-α(2-3)-Gal-β(1-3)-Gal*N*Ac-
Siglec-4b	SMP	Schwann cells in quail	Neu5Ac-α(2-3)-Gal-
Siglec-5		Granulocytes and monocytes	Neu5Ac-α(2-3/6)-Gal-

As mentioned above (**1.2.1.**), MAG belongs to the siglecs, a structurally and functionally related family of cell surface receptors that bind to sialic acid containing glycoproteins and glycolipids.[24] To date, as many as eleven members have been identified, including sialoadhesin (Sn, siglec-1), CD22 (siglec-2), CD33 (siglec-3), MAG (siglec-4a), Schwann cell myelin protein (SMP, siglec-4b), and siglec-5~11(*table* 1).[60,61] Each member contains two or more Ig-like domains: an amino-terminal V-set domain followed by one or more C2-set domains, a single transmembrane anchor, and a short cytoplasmic tail.[62,63] The ligand recognition site has been localized in the amino-terminal V-set domain or the V-set domain with

contributions from the adjacent C2-set domain (CD22).[64,65] The first two domains share very high amino acid sequence similarity between MAG and SMP (>70%), and significant similarity across all siglecs (>30% in pairwise comparisons).[62,66,67] Each siglec, by definition, recognizes a terminal sialic acid residue that is essential for binding, whereas significant differences in sialic acid linkage specificity are observed.[68] For example, sialoadhesin binds terminal α2,3- or α2,8-linked sialic acid,[69] CD22 recognizes solely α2,6-linked sialic acid,[70] and MAG requires α2,3-linked sialic acid, preferentially, a Neu5Acα(2→3)Galβ(1→3)Gal*N*Ac terminus as implicated by initial specificity study.[56] As already reported 30 years ago, gangliosides carry 75-80% of the sialic acids content in the brain.[58] Representative examples are the major brain gangliosides GD1a and GT1b (*figure* 5). In addition, these gangliosides abound on the neuronal cell surface and also along the axons, are placed directly apposed to MAG *in vivo*.[10] The presence of the MAG binding glycan sequence on gangliosides, and their location on the axon surface led to the hypothesis that gangliosides may be endogenous ligands for MAG, and may, therefore, mediate MAG's physiological functions.

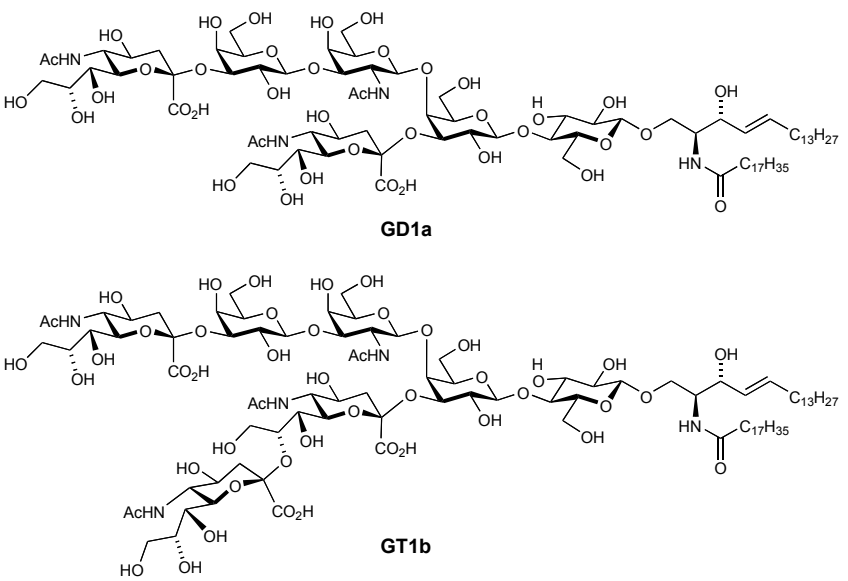


Figure 5: major brain gangliosides GD1a, GT1b.

Substantial data are consistent with this hypothesis. Firstly, direct binding study showed that MAG binds to GD1a, GT1b and related gangliosides with high specificity and affinity *in vitro*.[71] Secondly, the binding of MAG with gangliosides is blocked by mAb 513, a conformationally specific anti-MAG antibody that also blocks

MAG-neuron binding.[71] Finally, mice genetically lacking the Neu5Acα(2→3) Galβ(1→3)Gal*N*Ac terminus on gangliosides (but not on glycoproteins) suffer from axon degeneration and demyelination similar to that in MAG knockout mice.[72]

Furthermore, evidence from extensive studies demonstrated that gangliosides are both necessary and sufficient to support MAG-mediated neurite growth inhibition.[57]

• Treatment with *V. cholerae* neuraminidase, which converts the MAG-binding gangliosides GD1a and GT1b to the nonbinding GM1 (*table* 2, *entry 2*), eliminates MAG-mediated inhibition.[57]

• Gangliosides are synthesized by sequential addition of monosaccharides to ceramide by specific glycosyltransferases (*figure* 6). By treating with glucosylceramide synthase inhibitor 1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol (P4), which blocks synthesis of all glycosphingolipids without influencing cell viability or neurite outgrowth,[73] cell surface gangliosides were completely depleted. As a consequence, a significant reversal of MAG-mediated inhibition was observed with P4 treated neurons.[57] Furthermore, genetically engineered mice lacking the Gal*N*Ac transferase, which is required for the biosynthesis of the Neu5Acα(2→3)Galβ(1→3)Gal*N*Ac terminus, expressed compensatory amount of the simpler gangliosides GD3 or GM3 (*table* 2, *entries 1 and 4*) instead of GD1a and GT1b. Consequently, neurons from these mice showed attenuated response to MAG-mediated inhibition.[57]

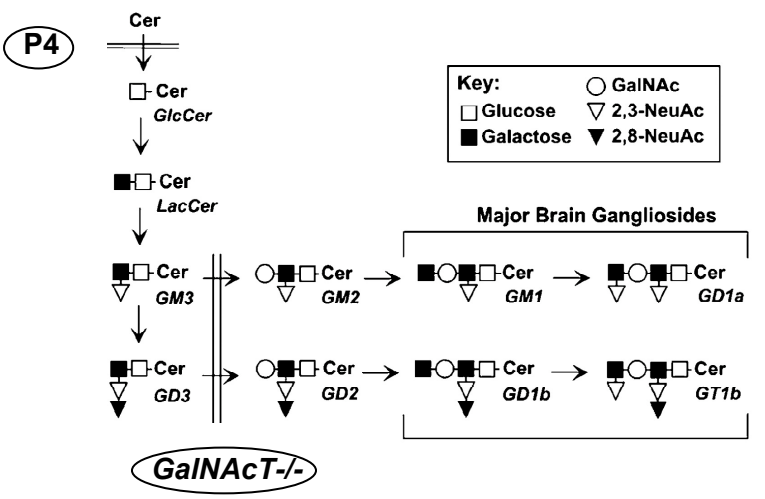


Figure 6: The biosynthetic pathway for major brain gangliosides. Pharmacological (P4) and genetic (*GalNAcT* -/-) blocks in the pathway are indicated.

• Monoclonal antiganglioside Abs can block MAG-mediated inhibition of neurite outgrowth. Anti-GD1a Ab sharply reduced the inhibition, anti-GT1b Ab had an intermediate effect, whereas anti-GD1b or anti-GD3 Abs had no effects.[57]

• Multivalent Ab-induced clustering of gangliosides GD1a or GT1b mimics MAG-mediated neurite outgrowth inhibition without MAG's involvement, probably through a direct interaction with $P75^{NTR}$.[74] In contrast, precomplexed anti-GM1 IgG had no such effect.[57]

As a summary of the above, gangliosides containing the Neu5Acα(2→3) Galβ(1→3)Gal*N*Ac terminus, are not only highly necessary to support MAG-mediated neurite outgrowth inhibition, but also sufficient to induce the same inhibition by multivalent clustering. In addition, GT1b was found to have specific association with $P75^{NTR}$,[74] implicating that, besides MAG-NgR-$P75^{NTR}$, gangliosides and $P75^{NTR}$ may also form a receptor complex for MAG to transmit the inhibitory signals into neurons.

1.3.2. Structural specificities of gangliosides for MAG binding

Mammalian ganglioside-binding proteins have distinct structural specificities for their carbohydrate targets. One of the most highly specific ganglioside binding lectins is MAG.[56] To date, certain structure-function studies of MAG-mediated cell adhesion to gangliosides and related glycosphingolipids were carried out to elucidate structural specificities of MAG-recognized carbohydrate (*table* 2).[56,75,71]

Table 2: Adhesion of natural and modified gangliosides to MAG-transfected COS cell.

Entry	Ganglioside	Conc. of ganglioside supporting half-maximal cell adhesion	
		[pmol/well]	[Ref.]
1	**Neu5Ac-α(2-3)**-Gal-β(1-4)-Glc-β-Cer **GM3**	>100[a]	71
2	Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer Neu5Ac-α(2-3) **GM1**	n.d.[b]	56,75
3	Neu5Ac-α(2-6) Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **GM1α**	n.d.	56,75

4	**Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **GM1b**	80	56,75
5	Neu5Ac-α(2-8)-Neu5Ac-α(2-3)-Gal-β(1-4)-Glc-β-Cer **GD3**	n.d.	71
6	Neu5Ac-α(2-6) **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **GD1α**	19	56,75
7	**Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer Neu5Ac-α(2-3) **GD1a**	50	56,75
8	Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer Neu5Ac-α(2-8)-Neu5Ac-α(2-3) **GD1b**	n.d.	71
9	Neu5Ac-α(2-6) **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer Neu5Ac-α(2-3) **GT1aα**	17	56,75
10	Neu5Ac-α(2-8)-Neu5Ac-α(2-6) **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **GT1β**	50	56
11	**Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer Neu5Ac-α(2-8)-Neu5Ac-α(2-3) **GT1b**	50	56,75
12	Neu5Ac-α(2-8)-Neu5Ac-α(2-6) Neu5Ac-α(2-3)-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer Neu5Ac-α(2-8) **GQ1β**	n.d.	56
13	Neu5Ac-α(2-3)-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer Neu5Ac-α(2-8) Neu5Ac-α(2-8)-Neu5Ac-α(2-3) **GQ1b**	n.d.	56
14	Neu5Ac-α(2-6) **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer Neu5Ac-α(2-8)-Neu5Ac-α(2-3) **GQ1bα**	6.0	56,75
15	**Neu5Ac-α(2-3)**-Gal-β(1-3)-**Glc*N*Ac**-β(1-4)-Gal-β(1-4)-Glc-β-Cer	240	56,75
16	**Neu5Ac-α(2-3)**-Gal-β(1-**6**)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer	87	56,75
17	**HO_3S-(6)** **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer	22	56,75
18	**HO_3S-(6)** **Neu5Ac-α(2-3)**-Gal-β(1-**4**)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **HO_3S-(3)**	1.5	56,75

[a] low but statistically significant adhesion over background.
[b] no detectable adhesion at >100 pmol/well.

Firstly, data in *table* 2 show, that MAG only binds to gangliosides containing a terminal α2,3-linked sialic acid (compare GM1 to GD1a, GD1b to GT1b, *entries 2, 7, 8 and 11*), but the adhesion was abrogated when it is capped by a α2,8-linked sialic

acid (compare GT1β to GQ1β, GT1b to GQ1b, *entries 10, 12, 11 and 13*).[56] Secondly, it is notable that an unique quantitatively minor family of gangliosides termed "Chol-1" gangliosides, including GQ1bα (*figure* 7), GT1aα, GD1α, and GM1α (*entries 14, 9, 6 and 3*), displays enhanced potency for MAG-mediated adhesion except GM1α (GQ1bα > GT1aα, GD1aα > GT1b, GD1a > GM1b).[75] Chol-1 gangliosides are related to the major brain gangliosides but have an additional α2,6-linked sialic acid on the Gal*N*Ac of a gangliotetraose core, making them part of the "α-series" ganglioside family.[76-78] GQ1bα (0.5 mg/kg of brain) and GT1aα (0.9 mg/kg), defined as the two major Chol-1 gangliosides, are very minor species compared with GD1a, the major ganglioside of brain (1200 mg/kg).[76-78] Nevertheless, their high binding affinities for MAG demonstrated the contribution of the additional α2,6-linked sialic acid, which by itself does not support MAG binding (GM1α has no affinity).[56,75] GQ1bα, the most potent natural ganglioside identified so far, is about 10-fold more active in supporting MAG-mediated adhesion than GT1b, the closely related ganglioside lacking only the α2,6-linked sialic acid (*entries 14 and 11*).[56,75] Furthermore, the different affinity between GD1α and GD1a suggests that as an additional contributor, α2,6-linked sialic acid is more significant than α2,3-linked internal sialic acid, although both of them can enhance MAG-mediated adhesion (*entries 6 and 7*).[56,75] Last, in contrast to the marked binding effect of sialic acids attached on the neutral core, changes in the oligoaccharide core have only minor effects. The replacement of the core Gal*N*Ac with Glc*N*Ac (*entry 15*) reduced the binding affinity about 3-fold as compared to GM1b (*entry 4*), suggesting a modest impact on recognition.[56] Surprisingly, altering the Gal-Gal*N*Ac linkage from β1,3 to β1,6 (*entry 16*), which might be expected to have a large conformational effect, did not influence MAG binding by comparing with GM1b.[56] This is consistent with a study by Kelm, where modifications on either monosaccharide residue of the neutral core (*e.g.* 4/6-deoxy, 6-*O*-methyl, 6-NH_2 of Gal; various *N*-substituted glucosamines, Fuc instead of Gal*N*Ac) or the linkage between them did not cause significant difference for MAG-binding.[79] These data indicated that some tolerance for modifications of the neutral saccharide backbone. The core behaves more like a spacer to hold two sialic acids in the specific orientation needed for binding with MAG.

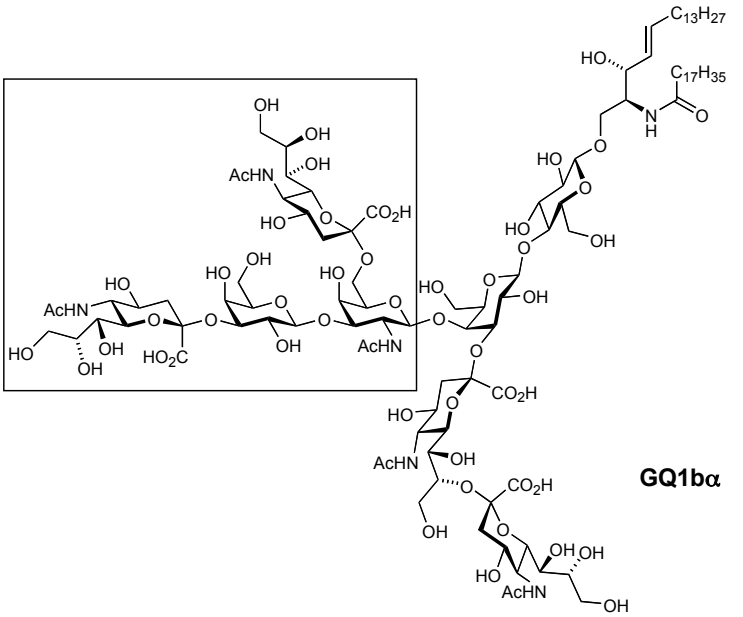


Figure 7: Ganglioside GQ1bα with tetrasaccharide "Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)] Gal*N*Ac" in box.

In summary, the α2,3-linked sialic acid on the terminal Gal is the primary determinant for MAG binding, while the additional α2,6-linked sialic acid on Gal*N*Ac can remarkably increase the binding affinity. Compared with the significant impacts of these two sialic acids, the disaccharide core is not comparably specific, which means that it may be not greatly involved in the protein-binding site. According to these observations from the above structure-function studies, tetrasaccharide Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)]Gal*N*Ac, which is a partial structure of GQ1bα, can be deduced as the minimal determinant structure for MAG binding with high affinity (*figure* 7, in box).

1.3.3. Sialic acids substructural specificities for MAG-binding

Sialic acids occur on cell surfaces at exposed positions, mostly terminal components with different linkages to the glycans of glycoconjugates. A unique feature of sialic acids is their structural variability leading to more than 40 naturally occurring modifications. Therefore, sialic acids are thought to play important roles in cellular interactions.[80,81] As discussed before, the sialic acids of gangliosides are highly essential for MAG-binding. To further investigate the contributions of substructural features of sialic acid for MAG-binding affinity, chemically synthesized and modified gangliosides were employed for extensive structure-function study.

Research in this field was carried out mostly by two groups: Schnaar *et al.*[56,57,71,75,82] studied adhesion between natural and synthetic gangliosides adsorbed onto microwell plastic plates and full-length MAG expressed on the transiently transfected COS cell surface; Kelm *et al.*[79,83] employed "heptan inhibition assay" which observed the binding between Fc-chimera containing *N*-terminal 3 or 5 domains of MAG (Fc-MAG_{1-3} or Fc-MAG_{1-5}) and soluble natural and synthetic gangliosides in the presence of human erythrocytes. Due to the different assays, some conflicting data were reported by these two groups. However, most results are consistent, or supplementary with each other, which made the picture of sialic acid substructural specificities for MAG binding more and more clear.

α2,3-linked terminal sialic acid — Sia(I)

When GD1a is modified at the carboxylic acid by esterification, amidation, or reduction, MAG-binding is abolished, strongly demonstrating that the anionic charge is crucial for protein binding (data not shown).[71] Additionally, this carboxylic acid needs to be in axial position, since only α-anomer, the natural conformation in sialylated glycans support MAG-binding.[83] It is in good agreement with the X-ray structure of the *N*-terminal immunoglobin-like domain of the sialoadhesin in complex with 2,3-sialyllactose, which shows the axial carboxylic acid of the terminal sialic acid directedly involved in binding by forming a salt bridge with Arg97.[84] It should be noted that this Arg is highly conserved in the first Ig-domain of other members of siglec family, while Arg97 in sialoadhesin corresponds to Arg118 in MAG.[35]

The glycerol side chain of sialic acid was shown to be crucial for binding in Schnaar's studies with modified GD1a. The binding affinity to MAG was abolished with truncated glycerol chain, 7/8-aldehydes, 7/8-alcohols, as did deoxy and/or methoxy derivatives of the 7, 8 or 9- position of Sia(I) of GM3 (data not shown).[71,82] These results suggested that the intact glycerol chain is needed for binding and that eliminating or derivating any of the OHs in this part reduced binding. The role of 9-OH attracts the interest based on the X-ray of sialoadhesin cocrystallized with sialyllactose, which implicates that an additional contact with the protein at that place might be exist.[84] From Kelm's study of a series of methyl sialosides, additional

structural information was obtained demonstrating the requirement of 9-OH for binding (*table* 3).[83] Replacement of 9-OH with hydrogen (*entry 3*) or halogens (*entries 4 to 6*) did not support binding, while a 9-NH_2-analogue (*entry 8*) showed a 3-fold increase in binding, suggesting 9-OH functions as a hydrogen bond donor for one or more amino acids in the binding pocket. The introduction of a thiol group resulted in much lower affinity (*entry 7*), because SH forms weaker hydrogen bonds with the protein, and the steric constraints by the large sulfur atom may also be the reason. More recently, a systematic study based on C(9)-NH_2 was carried out by differently substituting the amine.[85] Notably, acyl groups such as benzoyl, biphenylcarbonyl, naphthylcarbonyl (*entries 9 to 11*) increased the binding dramatically. Among them, methyl-α-9-*N*-benzamido-9-deoxy-Neu5Ac enhanced the binding as much as 700-fold compared with the reference (*entry 1*). This indicates indeed an additional hydrophobic contact with the Tyr 44, 46 residues of MAG.[86]

Table 3: Relative inhibitory potencies (rIPs) of modified methyl sialosides for MAG

Entry	Compound	rIP	[Ref.]
1	Neu5Ac-α-Me	1.00	83
2	Neu5Ac-α-Bn	9.80	83
3	9-deoxy-Neu5Ac-α-Me	n.a.	83
4	9-Cl-Neu5Ac-α-Me	n.a.	83
5	9-Br-Neu5Ac-α-Me	n.a.	83
6	9-I-Neu5Ac-α-Me	n.a.	83
7	9-thio-Neu5Ac-α-Me	n.a.	83
8	9-NH_2-Neu5Ac-α-Me	2.98	83
9	9-benzoyl-NH-Neu5Ac-α-Me	704	85
10	9-bipheyl-4-carbonyl-NH-Neu5Ac-α-Me	218	85
11	9-naphthyl-2-carbonyl-NH-Neu5Ac-α-Me	236	85
12	Neu5Propyl-α-Me	1.56	83
13	Neu5aminoAc-α-Me	n.a.	83
14	Neu5ThioAc-α-Me	3.85	83
15	Neu5FAc-α-Me	16.94	83
16	Neu5ClAc-α-Me	7.00	83
17	Neu5F_3Ac-α-Me	4.04	83

n.a. not applicable since less than 50% inhibition at the highest concentration tested.

Concerning the modifications of the *N*-acetyl group at C5, R.L. Schnaar reported that GM3 bearing *N*-glycolyl neuraminic acid (NeuGc), which is rare in humans but common in rodents, did not support adhesion.[82] Furthermore, GM4 derivatives bearing a 5-deaminated neuraminic acid (KDN-GM4) failed to support binding as well (data not shown).[71] Both results clearly demonstrated that modifications at 5-position are not tolerated by the protein. Based on the X-ray structure of sialoadhesin cocrystallized with sialyllactose showing that a Trp residue in the binding site interacts specifically with the methyl group of the acetyl moiety, Kelm *et al.* investigated this position in much detail.[83] Except the contradictory finding about the terminal KDN of a pentasaccharide, which bind to MAG 6.47 times stronger than its NeuAc analogue (data not shown), Kelm's investigation confirmed the crucial role of *N*-acetyl group for recognition. In a study where propionyl (*entry 12*), amionacetyl (*entry 13*), thioacetyl (*entry 14*) or halogenated acetyl groups (*entries 15-17*) were compared with the acetyl residue, MAG showed significant enhancement of the binding affinity with halogenated acetyl groups. It should be noted that *N*-fluoroacetyl sialic acid derivative (*entry 15*) is bound about 17-fold better than the reference, while *N*-chloroacetyl and *N*-trifluroacetyl analogues increased affinities with 7- and 4-fold, respectively (*entries 16 and 17*). The enhanced affinity can be rationalized by halogen mediated additional contacts with the protein or by electronic effects on the amide, which results in a weaker hydrogen bond acceptor quality of the carbonyl oxygen and a significantly increased hydrogen bond donor quality of the amino group.[83]

α2,6-linked internal sialic acid — Sia (II)

The increased potency of Chol-1 gangliosides, like GQ1bα, to support MAG-mediated adhesion suggests a potential direct interaction between the α2,6-linked internal sialic acid and the protein. Extended explorations of the substructural specificity of Sia(II) to further define the determinant for MAG binding are reported in the literature.[75]

To test the role of the anionic charge, the entire Sia(II) moiety of GD1a was replaced with a sulfate group (*table* 2, *entry 17*) which had only a small influence on MAG

binding, indicating negative charge to be the key pharmacophore in the Sia(II) moiety.[75] Notably, an analogue of GT1aα bearing two sulfate groups instead of two sialic acid moieties showed a 10-fold increase in binding affinity for MAG, compared with GT1aα (*entries 9 and 18*).[75] Unlike for Sia(I), modifications of the exocyclic glycerol chain (GD1α bearing a 7-, 8- or 9-deoxy) supported MAG-mediated adhesion, suggesting that the OHs on this exocyclic glycerol chain are not strictly required for enhanced recognition.[75]

In summarize, numerous evidence showed that gangliosides are MAG ligands. Furthermore, they are both necessary and sufficient to support MAG-mediated neurite outgrowth inhibition. Structure-activity relationship (SAR) studies with natural gangliosides suggest to focus on the tetrasaccharide terminus Neu5Acα(2→3) Galβ(1→3)[Neu5Acα(2→6)]Gal*N*Ac for high binding affinity with MAG, where both sialic acids are essential for recognition, while the Galβ(1→3)Gal*N*Ac core seems to act predominantly as a linker (*figure* 8). Extended investigation of substructural specificity of Sia (I), Sia (II), mapped the epitopes important for binding: the intact Sia (I) moiety is necessary for binding, while modification at 5-, 9-positions can greatly enhanced the affinity; the anionic charge is the key point for the interaction of the Sia (II) moiety.

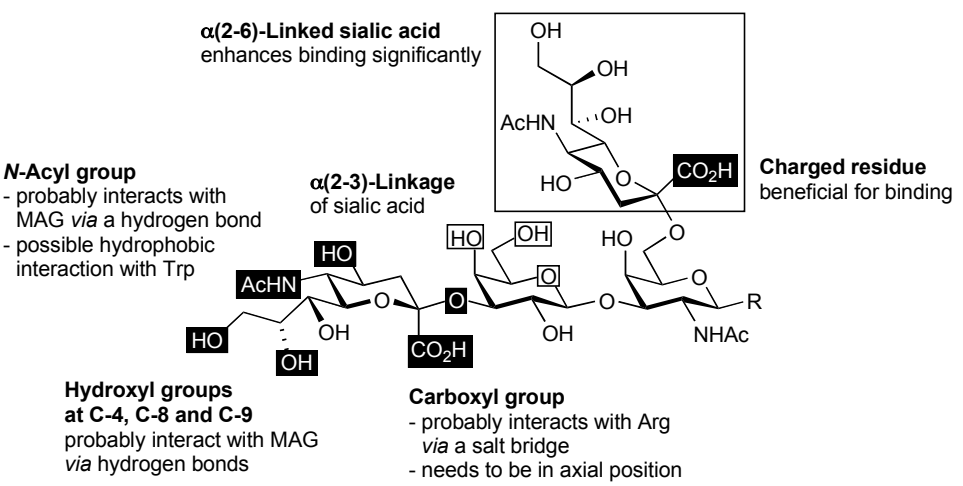


Figure 8: Structural specificities of tetrasaccharide terminus Neu5Acα(2→3)Galβ(1→3) [Neu5Acα(2→6)]Gal*N*Ac for MAG-binding.

2. Drawbacks of carbohydrates to be drugs

Carbohydrates are a major focus of current biological research. By interactions with proteins, carbohydrates play an important role in diverse cellular recognition and signaling events, such as cellular growth, adhesion, bacterial and viral infections, cancer metastasis, inflammation, and immune surveillance.[87] As a result, the development of carbohydrate-based therapeutics appears to have great potential for the treatment of myriad human disease. However, there is a series of obstacles that make carbohydrates a problematic class of lead structures for drug development, *e.g.* complicate carbohydrates synthesis, poor bioavailability, and low binding affinity for target proteins.[88-90]

2.1. Synthetic difficulties

Of the three major classes of biomolecules—proteins, nucleic acids, and carbohydrates—carbohydrates are the least exploited, which is due to the structural complexity resulting from the various regio- and stereo-specific linkages of oligosaccharides. The degree of molecular diversity generated from glycosidic linkage assembly is enormous: more than 10 million tetrasaccharides can be assembled from the 9 common monosaccharides, compared with 256 tetranucleotides and 16,000 tetrapeptides from the corresponding 4 nucleotides and 20 amino acid building blocks, respectively.[89] Moreover, to synthesize peptides or oligonucleotides, automated synthesizers are available for an iterative formation of a single bond type (peptide or phosphodiester bond respectively). However, synthesis of specific glycosidic linkage is much more difficult, as carbohydrates are densely functionalized with hydroxyl groups of similar reactivity. Laborious protecting-deprotecting manipulations make long overall synthetic routes. Despite recent advances in solid phase synthesis,[91-93] efficient regio- and stereoselective synthesis of oligosaccharides remains a challenge.

2.1. Low bioavailability

Many natural saccharides are rapidly degraded *in vivo*. In the stomach and in the intestine most of the glycosides are hydrolyzed, either by the action of the acidic environment (stomach) or by the action of glycosidases (small intestine).[94] This is the reason that oligosaccharides are generally not stable enough to allow oral administration. In addition, glycosidases are presented also in other body fluids which can reduce a carbohydrate-based drug's half-life to just a few minutes, depending on its structure.[90]

One of the best-investigated examples is sialyl Lewis^x (sLe^x), a terminal tetrasaccharide of cell surface glycoproteins and glycolipids. It is a ligand for E-selectin, which mediates the early stage of inflammatory reactions or metastasis.[95-97] Though sLe^x has been considered to be potentially useful as an anti-inflammatory agent, this natural oligosaccharide can only be applied intravenously for acute symptoms, as it is orally inactive.[98] Even though, it can be hydrolyzed by glycosidase in blood, moreover, the majority was observed to be rapidly excreted from renal due to its high water solubility.

2.3. Low binding affinity

Carbohydrate-protein interactions show typically low affinities: the dissociate constant is most often in the millimolar to micromolar range ($K_D \approx 10^{-3}$ to 10^{-6} M), while the general perception of the one required for pharmaceutical applications is much lower ($K_D \approx 10^{-9}$ M).[99]

X-ray crystallographic studies show that extracellular carbohydrate binding sites are generally shallow and solvent exposed.[99] In addition, the protein makes only few direct contacts, which are distributed over a large contact area to the ligand. Hydrogen bonding is expected to play an important role in carbohydrate-protein recognition processes. Since the directionality of hydrogen bonds is critical, they contribute predominantly to the specificity of carbohydrate recognition. Their energetic contributions to carbohydrate-protein binding are rather small and have been extensively debated.[99,100,101]

Besides, the lack of hydrophobic groups, which are often dominant in high-affinity receptor-ligand interactions in the carbohydrate moieties, might also be responsible for the low affinity, although weak hydrophobic interactions between non-polar surface of sugars (*i.e.* α-face of β-galactose) and proteins have been identified. In addition, except certain anionic groups containing saccharides such as sLe^x, other sialic acid derivatives, and heparin, Coulomb interactions which stabilize the complex by ion pair or direct hydrogen bond contacts with the protein is missing in most carbohydrate-protein interactions.[99]

By considering entropy, the binding of most carbohydrates to proteins is an entropically unfavorable process due to the conformational flexibility of oligosaccharides. It is difficult to dissect the entropy costs on ligand binding from other thermodynamic parameters. However, the conformational restriction of inter-saccharide rotational freedom upon binding has been estimated to be as large as 1-2 kcal/mol.[99]

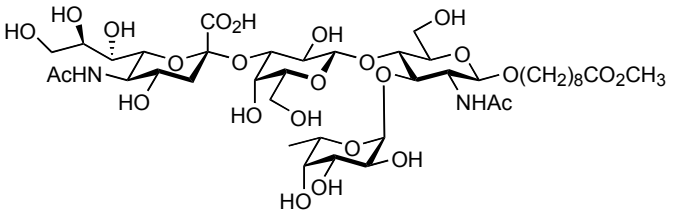
It should be noted that as both a hydrogen bond donor and acceptor, water is greatly involved in carbohydrate-protein binding, which is demonstrated by numerous X-ray crystallographic studies.[99,100] Its energetic impetus for complex formation has been debated, and Lemieux has argued that the driving force for carbohydrate complexation is the reorganization of water molecules upon binding.[102] For example, as revealed by the X-ray structure of *Griffonia simplicifolin* lectin complexed to the lewis b tetrasaccharide α-L-Fuc(1→2)β-D-Gal(1→3)[α-L-Fuc(1→4)]β-D-Glc*N*Ac, 8 of 11 water molecules present in the binding site of the uncomplexed lectin are displaced by the ligand during binding.[100] The release of the more ordered structural water to bulk upon binding is enthalpically unfavorable but entropically favorable.

The thermodynamics of carbohydrate-protein interaction is determined by calorimetrically measured changes in enthalpies, entropies, free energies and molar heat capacity upon binding.[99,103] Virtually all the examples show free binding energies of 4-8 kcal/mol, corresponding to association constants of 10^3-10^5 M^{-1},[103] which confirms the low binding affinity of carbohydrate-protein interaction generally observed.

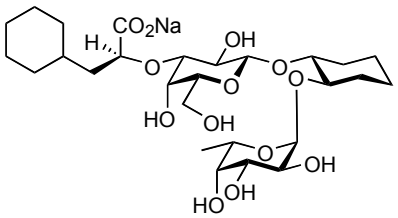
2.4. Carbohydrate mimics

A number of strategies were pursued to circumvent the carbohydrate-specific problems for drug discovery. Besides research on multivalent carbohydrate ligands that increase the affinity by multiple simultaneous binding events,[104-107] numerous studies have been focused on carbohydrate mimics.[88,108] There are two main strategies in the construction of carbohydrate mimics: Firstly, the substitution of the *O*-glycosidic bond with non-natural linkages or replacement of ring oxygen by other atoms and secondly, the replacement of the entire glycosidic scaffold with a small, non-carbohydrate framework bearing the required functional groups in the same spatial orientation as the parent sugar structure.[109] The former type of mimics,[110] such as *C*-glycosides, *S*-glycosides, carba-sugars, would ideally have increased resistance to endogenous enzymatic hydrolysis and chemical degradation, while conserving the global geometry of the native oligosaccharide. The second type of mimics is generally based on different core structures, and mimic the functions of lead sugars by keeping the essential functional groups required for recognition. Additionally, by virtue of the rational design based on structural details of the protein binding site, higher affinities and selectivities can be achieved by additional interactions with hydrophobic or charged groups of their cognate receptors.[88]

The second strategy described above is demonstrated with example from the selectin research. CGP69669 is a mimic of the tetrasaccharide sLe^x, the natural ligand for E-selectin (*figure* 9). Basing on the knowledge that anionic charge is the only pharmacopher of the terminal sialic acid, and the Glc*N*Ac behaves merely as a spacer to hold Gal and Fuc in the appropriate position, CGP69669 was designed in such a way that sialic acid was replaced by the much simpler cyclohexane L-lactic acid and Glc*N*Ac by cyclohexane 1,2-diol. The resulting IC_{50} was about 12-fold better than that of sLe^x from *in vitro* biological assay.[111]



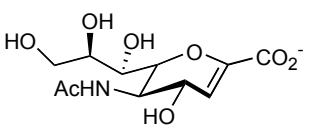
sLe^x; IC$_{50}$ = 1mM



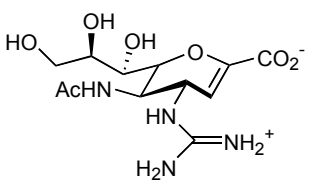
CGP 69669; IC$_{50}$ = 0.08mM

Figure 9: sLe^X and its mimic CGP 69669.

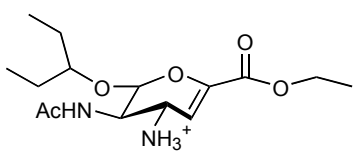
Another interesting example is the development of anti-influenza agents (*figure* 10).[112] Zanamivir was designed to mimic the transition state of sialic acid (Neu5Ac2en) in the hydrolysis reaction by neuraminidase. By keeping the carboxylic acid, the glycerol chain and *N*-acetyl function that are essential for the binding, the basic guanidinyl group was attached on 4-position, due to the strong interaction with Glu 119 and Glu 227 identified by X-ray crystal analysis.[112] Notably, K_i of Zanamivir is 1,000 times better than the parent compound Neu5Ac2en.[113] However, due to its poor bioavailability of only 2% ($logD_{7.4} \approx -6$), Zanamivir can not be applied orally. By further modification based on a prodrug concept, Oseltamivir with 78% bioavailability ($logD_{7.4} \approx 0.5$) and similar activity ($K_i \approx 1$ nM) was obtained, which is now on the market as an orally administered drug to block influenza virus infection.[114-116]



Neu5Ac2en $K_i \approx 1\mu M$



Zanamivir $K_i \approx 1nM$



Oseltamivir $K_i \approx 1nM$

Figure 10: Neu5Ac2en, Zanamivir and Oseltamivir at physiological pH.

3. Aim of this work

3.1. General strategy

The aim of MAG project—a collaboration of the Institute of Molecular Pharmacy, University of Basel (Prof. B. Ernst), University of Bremen (Prof. S. Kelm), University of Hamburg (Prof. B. Meyer) and University of Lübeck (Prof. T. Peters)—is to further define the structural requirements of the carbohydrate specificity for MAG, and in turn design and synthesize novel mimics with simplified structures and retained or even better binding affinities. The approach is based on the integration of results from various approaches:

- Partial structures and derivatives of high affinity natural ganglioside GQ1bα were chemically and chemo-enzymatically synthesized;
- The affinities of chemical entities to MAG were tested in a fluorescent hapten inhibition assay;
- STD-NMR was employed to identify the binding epitopes of the chemical entities;
- The bound conformation was deduce by trNOE-NMR and;
- A 3-D homology model for MAG based on the X-ray structure of sialoadhesin and cognate ligand illustrated the interaction between functional groups of ligand and the amino acid residues in the protein-binding site.

Results from this multidisciplinary study are expected to give guidance for the rational design of novel MAG antagonists.

The goal of this thesis is focused on the synthesis of MAG antagonists based on partial structure and derivatives of natural ligands and mimics thereof. As discussed above (**1.3.**), the tetrasaccharide Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)] Gal*N*Ac, which is a partial structure of GQ1bα, is the key determinant for MAG binding. In this thesis, besides trisaccharide Neu5Acα(2→3)Galβ(1→3)Gal*N*Ac, Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)]Gal and derivatives with Gal instead of Gal*N*Ac at the reducing end were chemically and chemo-enzymatically synthesized. In turn, according to the biological and NMR analyses, together with the previous

SAR studies, two groups of mimics focusing on the modifications on C-9 of Sia (I) and the disaccharide core respectively were designed and synthesized.

3.2. Chemical and chemo-enzymatic synthesis

Despite considerable recent progress, efficient regio- and stereoselective chemical synthesis of oligosaccharides remains a significant challenge. *In vivo,* oligosaccharides are synthesized by glycosyltransferases that sequentially transfer a single pyranosyl residue from a sugar nucleotide donor to a growing carbohydrate chain.[117] In contrast to the chemical synthesis, enzymatic synthesis using glycosyltransferases offers a number of advantages: high regio- and stereoselectivity, no requirement for chemical protection and deprotection and very mild reaction conditions.[118,119] The synthetic value of glycosyltransferases would be greatly enhanced, if their substrates tolerance would allow the glycosylation of non-natural substrates.[120] The combined approach of chemical and enzymatic synthesis, named chemo-enzymatic synthesis, would then greatly facilitate the preparation of complex carbohydrates and their mimics, which would increasingly provide insight into the biological roles of carbohydrates and initiate the rational design of new generations of carbohydrate based therapeutic agents.

It should be noted that stereoselective sialylation remains a major problem in carbohydrate chemistry, due to the sterically hindered tertiary anomeric center, the presence of an electron withdrawing carboxylate and the lack of a participating neighboring group in the 3-position of the sialic acid moiety.[121] Enzymatic synthesis employing sialyltransferases as biocatalysts offers an alternative approach with excellent stereoselectivity and generally good to excellent yield.[118,119] In this thesis, α(2→3)-sialyltransferase *ST3Gal III*[122-124]was used to transfer the sialic acid moiety from cytidine-5'-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) to the non-natural substrates.

3.3. Fluorescent hapten inhibition assay (in collaboration with Prof. S. Kelm, University of Bremen)

To analyze the inhibitory potentials of the partial structures of GQ1bα and derivatives and mimics thereof, a chimeric protein consisting of the Fc-portion of human IgG and the *N*-terminal 3 or 5 domains from MAG (Fc-MAG_{d1-3} or Fc-MAG_{d1-5}) was produced in COS cell and purified.[79,83]

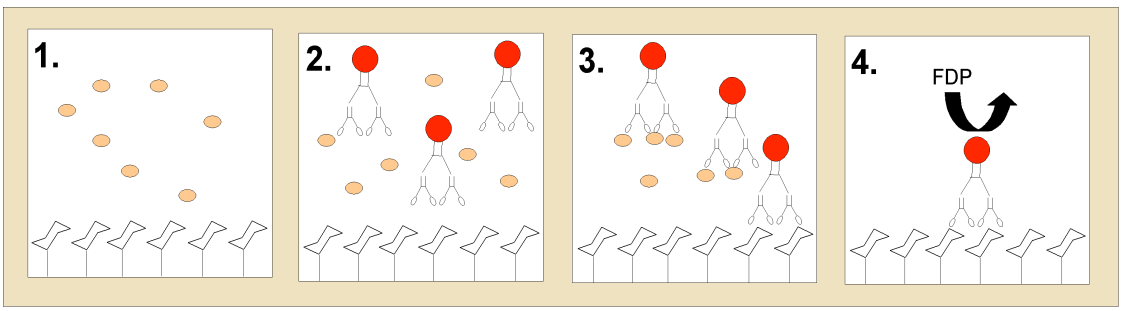


Figure 11: Fluorescent hapten inhibition assay. 1. Inhibitor was added to the sialic acid coated microtitre plates; 2. Fc-MAG complexed with polyclonal *goat anti human* IgG(Fc) coupled with alkaline phosphatase was subsequently added; 3. Incubation and removal of unbound complexes; 4. By treating with fluorescein diphosphate (FDP), the resulting fluorescence intensity that is proportional to the amount of bound complexes can be determined.

The fluorescent hapten inhibition assay (*figure* 11) employed in this project is a successor of the previous one which was based on radio-labeled MAG and erythrocytes as target cells.[79,83] In order to simplify the analyses, microtitre plates with covalently attached sialic acids instead of erythrocytes have been used as ligands for MAG. For the quantification of bound MAG, Fc-MAG is complexed with alkaline phosphatase-labeled anti-Fc antibody. During incubation, inhibitors occupy the protein-binding site and competitively inhibit the binding with the sialic acids coated plate. After washing for the removal of inhibitor-Fc-MAG complexes, the amount of Fc-MAG bound to the sialylated plates is determined by the initial velocity of fluorescein released from fluorescein phosphate. This method proved to be more reliable and easier to handle than the former assay relying on radiolabels. The inhibitory potencies obtained for several reference compounds were similar in both systems.

The concentrations required for 50% inhibition (IC_{50}) are determined from inhibition curves obtained from titration experiments. Since IC_{50}s may vary depending on the

condition from assay to assay, relative inhibitory potencies (rIPs) calculated by dividing the IC_{50} of the reference compound by the one of the tested compound were employed.[79,83] It is notable that rIP values are consistent, thus allowed a reliable comparison among different assays as long as the same reference is used.

All the compounds synthesized in the thesis have been analyzed in the fluorescent hapten inhibition assays using the same reference throughout the entire period.

3.4. STD NMR (in collaboration with Prof. B. Meyer, University of Hamburg)

The difference between a saturation transfer spectrum and a normal NMR spectrum provides a new and fast method, namely, saturation transfer difference (STD) NMR spectroscopy, to screen compound libraries for binding activity to protein, additionally, to map the epitopes of the protein binding ligand.[125]

By selective irradiating the protein with the pulse does not directly affect the resonances of small ligands, the saturation is spread over the entire protein by intramolecular saturation transfer. Ligand molecules interacting with the protein are saturated in turn by intermolecular transfer. Through chemical exchange these saturated ligands are transferred into solution where they are detected.[125] In addition, the ligand's binding epitope can be easily identified because ligand residues in direct contact of the protein show much stronger STD signals.

The intensity of STD NMR signal increases in accordance with the ligand excess and it is also correlated to the K_D of the ligand-protein binding, which should be between 10^{-3} and 10^{-8} M, in the range of the one from protein-carbohydrate interaction. Furthermore, there is no limit to the size of the protein. In fact, there is an increase of sensitivity with the size of protein due to a more efficient inter- and intramolecular saturation transfer.[126]

3.5. trNOE NMR (in collaboration with Prof. T. Peters, University of Lübeck)

It has been shown in many instances that oligosaccharides are flexible biomolecules.[127,128] Therefore, different conformational families normally coexist in solution. Upon binding to a receptor protein, one out of these numerous conformation is selected, which is called bioactive conformation. Knowledge of the bioactive conformation of various natural and artificial ligands is essential for a directed designing of the modified ligands that exploit the conformational control necessary for high binding affinity. For the analysis of bioactive conformation of oligosaccharides, NMR spectroscopy based on transfer nuclear overhauser (trNOE) effect is of fundamental importance. trNOE were originally observed and their principles described more than 20 years ago.[129] The experiment is based on a chemical exchange between ligand molecules in solution and ligand molecules bound to protein. One important physical parameter that distinguishes free and bound molecules is so called motional correlation or tumbling time τ_c.[130] τ_c is defined as the time that is required for a molecule to advance by one radian during translational and rotational diffusion. It is apparent that low or medium molecular mass molecules (<1-2 kDa) have a short τ_c and, as a consequence, exhibit positive NOEs, no NOEs, or very small negative NOEs depending on their molecular mass, shape and the field strength. When a small molecule is liganded with a large-molecular-mass protein, the relaxation is governed by the long τ_c of the protein, resulting in strong negative NOEs. Upon dissociation, the small ligand tumbles again with its own characteristic τ_c. Therefore, during a normal NOESY experiment, the large negative NOEs of the bound ligand will be transferred to the sharp signals of the free ligand. It is always the signal of the free ligand that carries the information about the bound state. trNOE allows an analysis of bioactive conformation without requiring any knowledge about the complex protein NMR spectrum. Additionally, trNOE experiments usually work at optimum with a 10-20 fold excess of ligands, making it very easy to observe signals of free ligand molecules against the background of broad protein signals.[130]

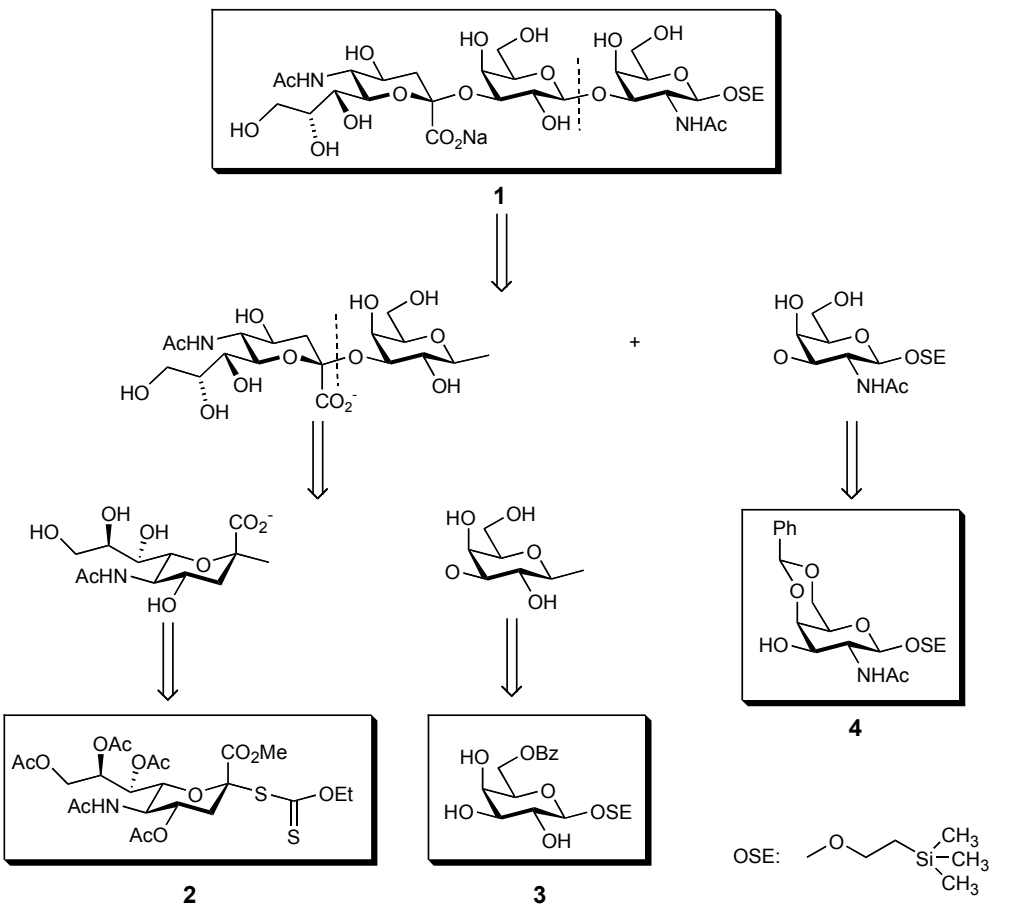
It is important to realize that trNOE is only observable for complexes with dissociation constant K_D approximately in the range between 10^{-6} to 10^{-3} M, since it is required that the dissociation of the complex is fast on the relaxation time scale. Consequently, trNOE is ideally suited for the investigation of protein-carbohydrate complexes that are usually characterized by low binding affinity.[130]

Results and Discussion

1. Partial structure of natural ligands and derivatives thereof

1.1. Synthesis of Neu5Acα(2→3)Galβ(1→3)Gal*N*Acβ-OSE (1)

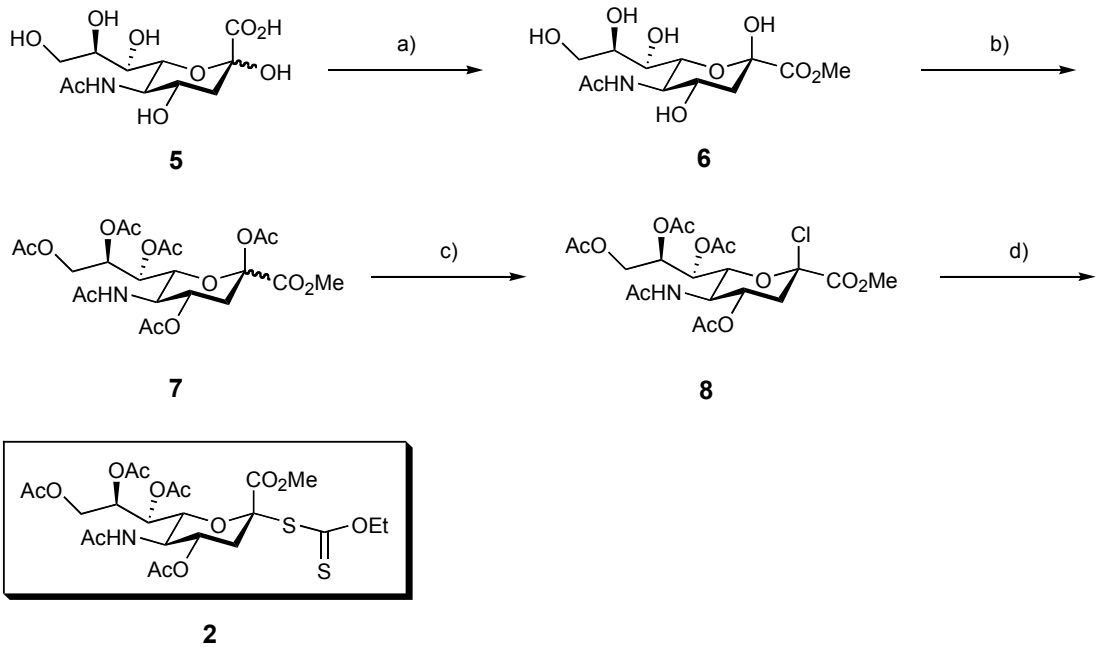
For the synthesis of Neu5Acα(2→3)Galβ(1→3)Gal*N*Ac terminus of GQ1bα and derivatives thereof, the 2-(trimethylsilyl)ethyl (OSE) group was chosen as the protecting group at the reducing end based on its compatibility of various reaction conditions used in carbohydrate chemistry.[131] According to the retro-synthesis depicted in *scheme* 1, the target molecule **1** was first dissected into Neu5Acα(2→3)Gal and Gal*N*Ac. The former was then divided into the two corresponding monosaccharides. For this purpose, three partially protected building blocks **2**, **3**, **4** were required.



Scheme 1: Retro-synthesis of Neu5Acα(2→3)Galβ(1→3)Gal*N*Acβ-OSE (**1**).

1.1.1. *O* -Ethyl *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]dithiocarbonate (2)

2-*O*-Ethyl dithiocarbonate derivative of *N*-acetylneuraminic acid (Neu5Ac), or sialyl 2-xanthate was selected as the sialyl donor because of the mild condition for its activation and its shelf stability.[132] Sialyl 2-xanthate derivative **2** was easily prepared in four steps from the commercial available Neu5Ac (**5**) as shown in *scheme* 2.[133]

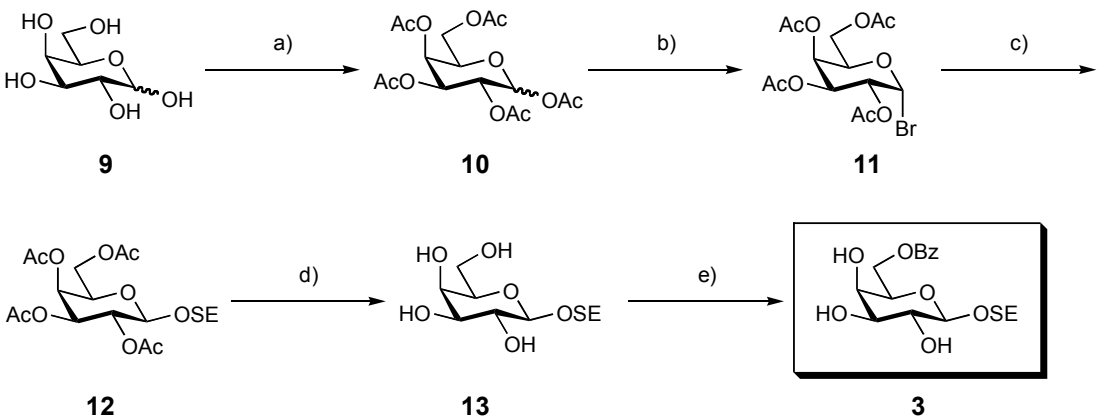


Scheme 2: a) *cat*. Amberlyst 15, MeOH, rt, 16 h (93%); b) Ac_2O, py, DMAP, rt, 48h (91%); c) AcCl, *conc*. HCl, CH_2Cl_2, rt, 16 h (77%); d) potassium *O*-ethyl dithiocarbonate, TBAHS, 5% aq. $NaHCO_3$, AcOEt, rt, 3 h (51%).

Esterification of Neu5Ac (**5**) with *cat*. Amberlyst 15 in MeOH afforded methyl ester **6** in 93% yield. Following acetylation with acetic anhydride (Ac_2O) in py and *cat*. amounts of 4-dimethylaminopyridine (DMAP) gave fully protected sialyl derivative **7**. The subsequent transformation to 2-β-chloro derivative **8** was achieved in 77% yield using acetyl chloride (AcCl), *conc*. HCl in a "Bombenrohr", a procedure originally reported by R. Kuhn *et al*.[134] The final displacement of 2-chloro substituent by *O*-ethyl dithiocarbonate with tetrabutylammonium hydrogensulfate (TBAHS) in *aq*. $NaHCO_3$-AcOEt proceeded via a SN_2 mechanism (→ **2**, 51%).[133]

1.1.2. 2-(Trimethylsilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside (3)

Based on the order of reactivity of the hydroxyl groups in galactose (primary hydroxyl is much more reactive than secondary hydroxyls; 3-OH is mostly more reactive than 2-OH, while the 4-OH is the least reactive one), building block **3** was designed as shown in *scheme* 3: benzoyl protection at 6-OH and OSE at the anomeric center. OSE was chosen not only for its stability under various conditions, but also because it is amenable to selective removal or transformation to other activated functional groups for further glycosylation.[131]

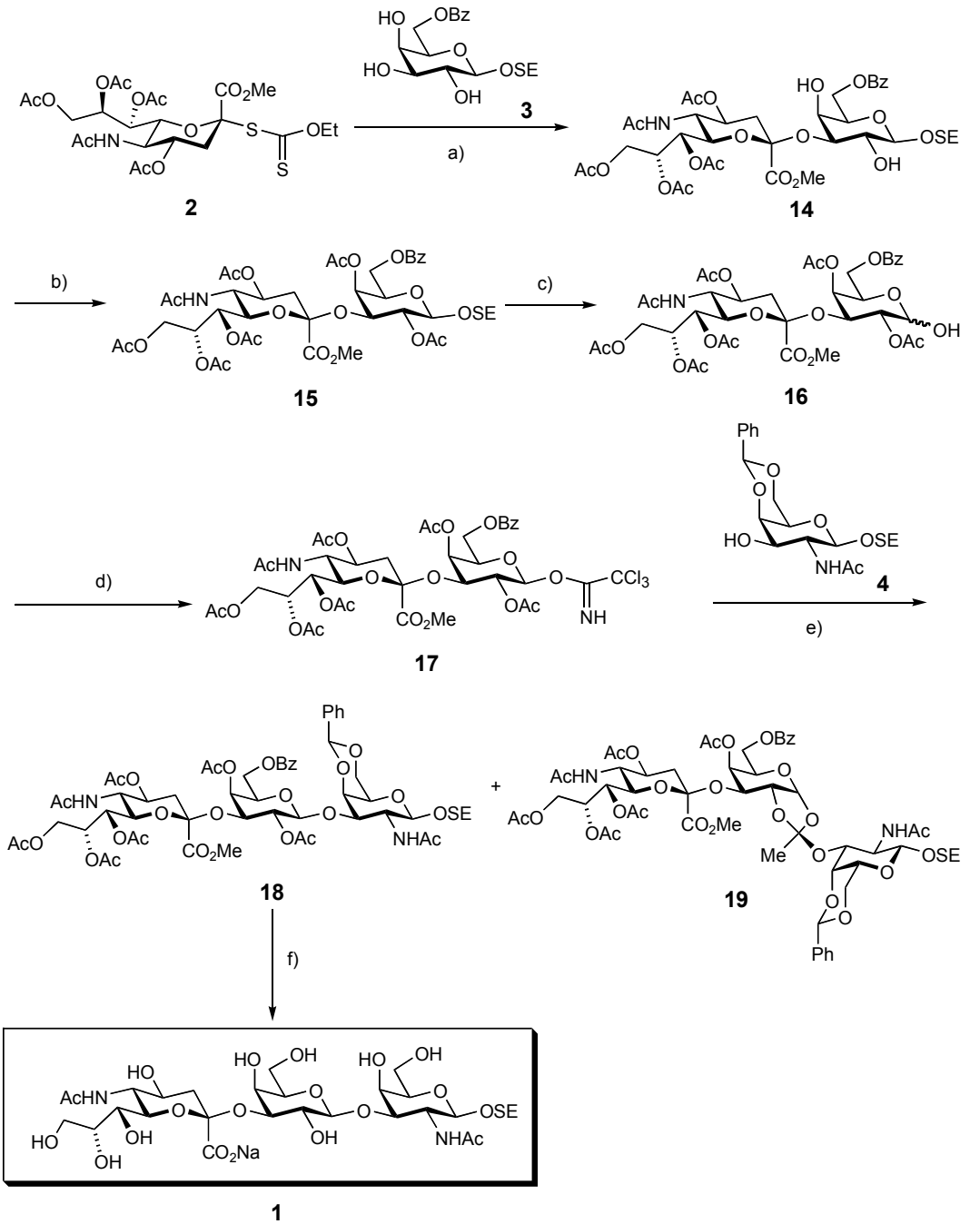


Scheme 3: a) Ac_2O, py, 0°C, 8 h, rt, 16 h (92%); b) 30% HBr-AcOH, CH_2Cl_2, 0°C, 2 h (87%): c) HOSE, HgO, $HgBr_2$, $CaSO_4$, toluene, rt, 48 h (91%); d) NaOMe, MeOH, rt, 2 h (98%); e) benzoyl cyanide, CH_3CN-Et_3N, -30°C, 2 h (56%).

By treating D-galactose (**9**) with Ac_2O in py, per-acetylated D-galactose **10** was formed quantitatively. Bromination[135,136] in 30% HBr in AcOH afforded **11** with exclusive α-configuration as confirmed by ^{1}H NMR ($J_{1,2}$ = 4.0 Hz) as a consequence of the "anomeric effect". Subsequent glycosylation of 2-(trimethylsilyl)ethanol (HOSE) was accomplished in the presence of HgO, $HgBr_2$ and dry $CaSO_4$ to yield the β-galactoside **12** ($J_{1,2}$ = 7.8 Hz) *via* S_N2 process.[131] After deprotection under Zemplén conditions, selective 6-*O*-benzoylation was reported either by a three step procedure involving 3,4-*O*-isopropylidenation of **13**, selective 6-*O*-benzoylation and subsequent de-*O*-isopropylidenation (54%),[137] or by the selective 3-*O*-benzylation of the 3,4-*O*-stannylene acetal followed by selective 6-*O*-benzoylation and finally hydrogenolysis to remove the benzyl group (36%).[138] With benzoyl cyanide in CH_3CN-Et_3N (4:1) at –30°C the 6-*O*-benzoylated product **3** was obtained in 56% yield. Compared to the common regents for benzoylation as benzoyl chloride or benzoic anhydride, benzoyl cyanide reacts smoothly with alcoholic, phenolic and mercapto functions with or without base catalysis, depending on the character of the respective function.[139] In the

presence of a catalytic amount of a base, benzoylated products are formed with high regioselectivity. The procedure is therefore extremely useful for the selective benzoylation of the multifunctional molecules, such as carbohydrates, nucleosides and nucleotides.[139,140]

1.1.3. Neu5Acα(2→3)Galβ(1→3)Gal*N*Acβ-OSE (1)

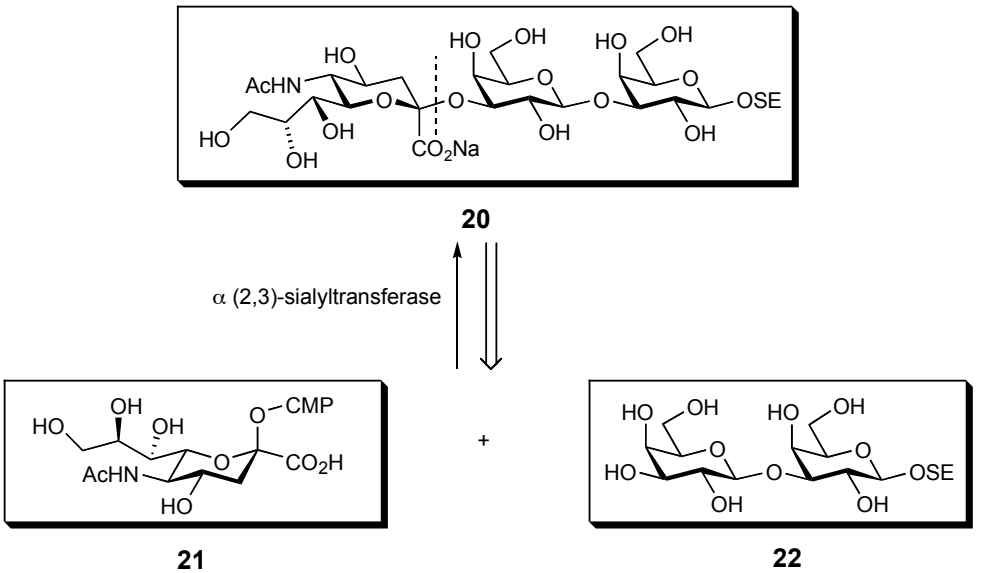


Scheme 4: a) NIS, TfOH, CH_3CN-CH_2Cl_2 (3:2), -70°C, 18 h (29%); b) Ac_2O, py, rt, 16 h (64%); c) BF_3-Et_2O, CH_2Cl_2, 0°C, 6 h (84%); d) CCl_3CN, Cs_2CO_3, CH_2Cl_2, 0°C, 8 h (74%); e) TMSOTf, CH_2Cl_2, 0°C, 4 h (**18**, 13%; **19**, 46%); f) i. 80% aq. AcOH, 45°C, 4 h, ii. NaOMe, MeOH, rt, 4 h, iii. aq. NaOH, rt, 16 h (50%).

The coupling reaction of sialyl donor **2** and acceptor **3** was carried out at –70°C with *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) as promoters[141,142] in CH_3CN-CH_2Cl_2 (3:2), affording the corresponding α-sialoside **14** in only 29% yield, while the amount of traces of β-anomer was not determined. After acetylation of the remaining hydroxyls, the reducing end was selectively deprotected by BF_3-Et_2O in CH_2Cl_2,[143] generating the hemiacetal saccharide **16** as an α/β-mixture. Subsequent introduction of the trichloroacetimido group at the anomeric center was achieved with trichloroacetonitrile in the presence of Cs_2CO_3 at 0°C in 74% yield. **17** was obtained mainly as β-anomer as indicated by ^1H NMR, which was in accordance with the literature data (α:β=1:8).[137] The following coupling reaction with acceptor **4** was first carried out by using BF_3-Et_2O as promoter in CH_2Cl_2 at 0°C. Instead of the expected product **18**, only ortho-ester **19** was detected. The similar result was observed when the reaction was promoted by trimethylsilyl trifluoromethanesulfonate (TMSOTf) in CH_2Cl_2 at -20°C, however, when the reaction took place at 0°C with the same condition, except **19**, trisaccharide **18** as a minor product was formed in 13% yield. Subsequent cleavage of its benzylidene group in 80% aq. AcOH, followed by transesterification with NaOMe in MeOH and hydrolysis in aq. NaOH afforded the target trisaccharide **1** in 50% yield.

1.2. Chemo-enzymatic synthesis of Neu5Acα(2→3)Galβ(1→3)Galβ-OSE (20)

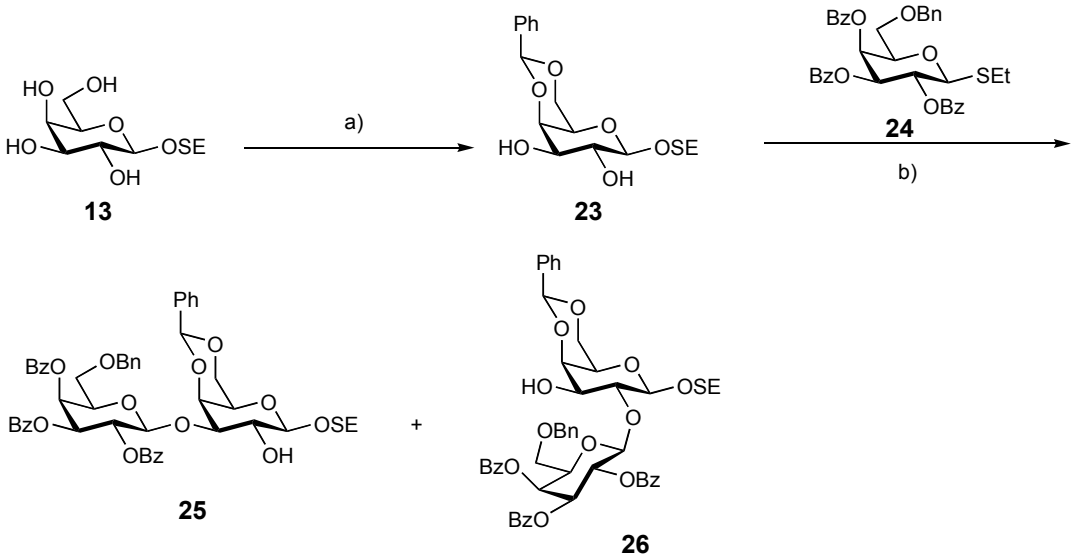
Based on the SAR studies of gangliosides for MAG binding, the internal Gal*N*Ac residue is not crucial for the binding affinity as compared to the terminal Neu5Acα(2→3)Gal disaccharide.[75] With a variety of monosaccharide moieties (Glc*N*Ac, Fuc), and/or with the different linkage patterns (Galβ1→4Glc*N*Ac, Galβ1→6Gla*N*Ac), no significant effect on MAG recognition was observed.[75,79] Trisaccharide **20** was therefore designed where Gal replaces Gal*N*Ac, which was supposed to have comparable affinity as **1** but would be much more readily accessible from the synthesis point of view. α(2→3)-Sialyltransferase (*ST3Gal III*, EC 2.4.99.6), which can regio- and stereoselectively transfer the sialic acid moiety to the 3-OH of the termial D-galactose,[118,144] offers an elegant approach for the synthesis of **20** (*scheme* 5).



Scheme 5: Retro-synthesis of Neu5Acα(2→3)Galβ(1→3)Galβ-OSE (**20**).

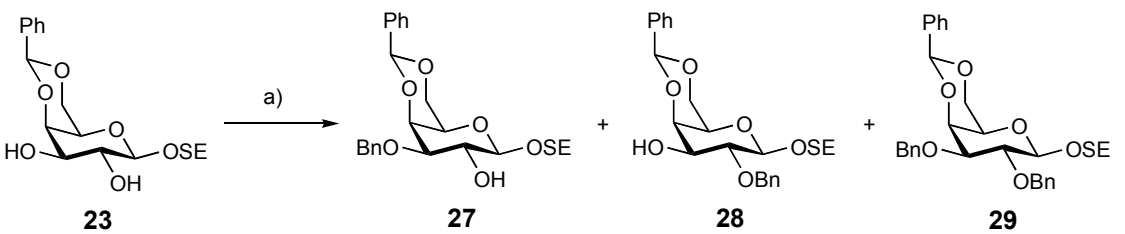
1.2.1. Chemical synthesis of Galβ(1→3)Galβ-OSE (22)

Galβ(1→3)Galβ-OSE (**22**) was initially designed to be chemically synthesized by the coupling of partially protected galactosyl acceptor **23** with galactosyl donor: ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside[145] (**24**), as shown in *scheme* 6. Galβ-OSE (**13**) was treated with α,α-dimethoxytoluene in CH_3CN in the presence of *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$) as catalyst, affording the 4,6-*O*-benzylidene derivative **23** in 88% yield. With the galactosyl donor **24**, the coupling reaction was carried out under standard condition: NIS-TfOH as the promoter in CH_2Cl_2 at –30°C. Surprisingly, the glycosylation took place at both 2- and 3-position of the acceptor (53%, **25**:**26** = 1:3), with the 2-coupled disaccharide **26** as the major product.



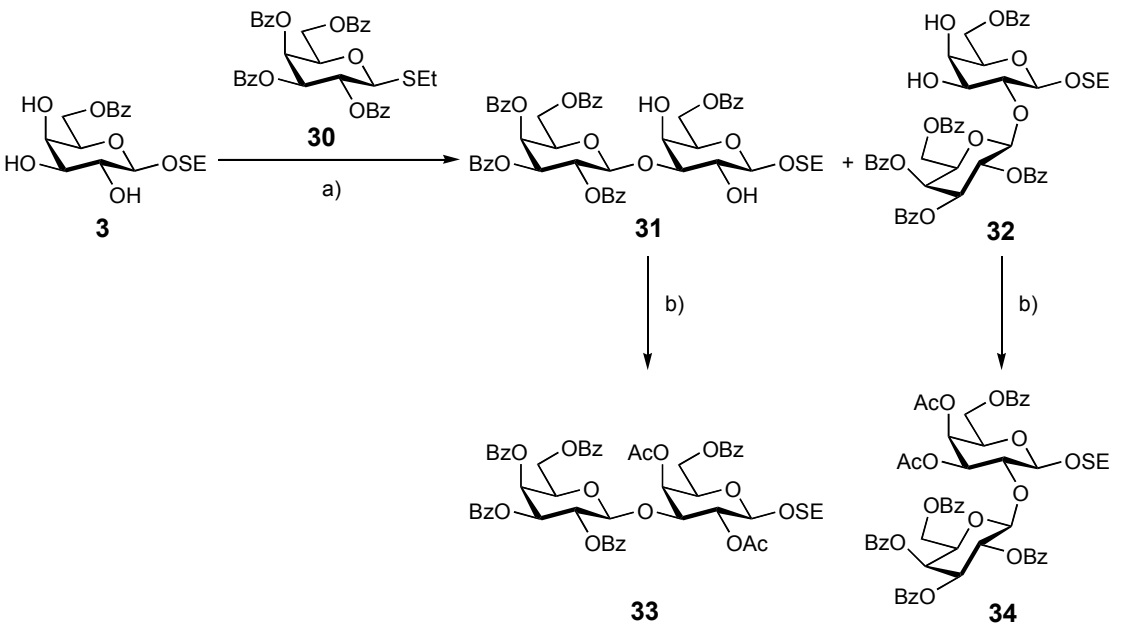
Scheme 6: a) α,α-dimethoxytoluene, $TsOH \cdot H_2O$, CH_3CN, 0°C, 1.5 h (88%); b) NIS, TfOH, CH_2Cl_2, -30°C, 1.5 h (53%, **25**:**26**=1:3).

Additional experiments were executed to study the influence of the reaction temperature and the amount of promoters on the regioselectivity. It was observed that by either lowing down the temperature to –80°C, or decreasing the promoter to half amount (1, 0.1 equiv. of NIS, TfOH respectively to donor **24**), byproduct **26** was predominantly formed together with **25** as indicated by TLC after 5 min., which implicated that 2-coupling product is both kinetically and thermodynamically favored. In contrast, as a general principle, 3-OH in 4,6-*O*-benzylidene-β-D-galactopyranoside is mostly found to be more reactive than 2-OH, because the former is less influenced by the electron-withdrawing effect of the anomeric center, hence is more nucleophilic than 2-OH. To confirm the reactivity difference between the two hydroxyls, simple benzylation was carried out under phase transfer condition, with benzyl bromide, tetrabutylammonium bromide (TBAB) in 5% aq. NaOH-toluene (*scheme* 7). In good agreement with the literature,[131] 3-benzylated product **27** was obtained as the major product in 63% yield, while 2-benzylated product **28** (13%) and 2,3-dibenzylated product **29** (4%) were also identified.



Scheme 7: a) benzyl bromide, TBAB, 5% aq. NaOH, toluene, 60°C, 22h (**27**, 63%; **28**, 13%; **29**, 4%).

Besides the different reactivity of hydroxyls, it is well known that regioselectivity also depends largely on the steric effect. The bulky substitution (4,6-*O*-benzylidene) might result in steric hindrance around 3-OH, which accounts for the reversed regioselectivity observed. To address this question, galactosyl donor ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside[146] (**30**) was coupled with 6-*O*-benzoylated galactosyl derivative **3** under the identical conditions (*scheme* 8). Again, the β(1,3)-linked product **31** was obtained as the minor component with a ratio of 1:3 in favor of the β(1,2)-linked product **32**. Since signals of H-2 and H-3 were overlapped in the ^{1}H NMR spectra of both products, the structures were confirmed after acetylation, leading to products **33** and **34** which exhibited a significant low field shift for the protons at the 2- or 3-position, respectively (H-2 of **31**→**33**: 3.75→5.16 ppm; H-3 of **32**→**34**: 3.71→4.99 ppm). Therefore, the influence of steric hindrance from 4,6-*O*-benzylidene substituent to the regioselectivity of glycosylation can be excluded.

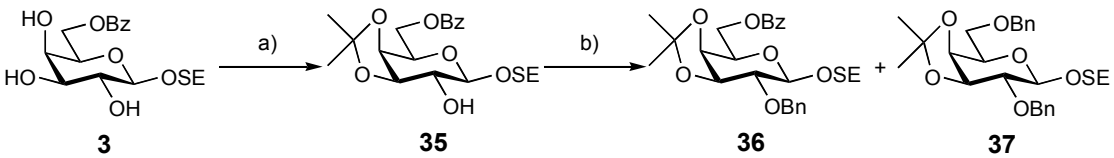


Scheme 8: a) NIS, TfOH, CH_2Cl_2, -30°C, 3 h (36%, **31**:**32**=1:3); b) Ac_2O, py, rt, 16 h (**33**, 90%; **34**, 90%).

Taken together, although 3-OH is more reactive than 2-OH in β-D-galactose moiety, coupling reactions took place primarily at the 2-position of the acceptors **23** and **3**, independent of a bulky substituent at the 4-position. A possible reason might be the bulky donors **24** and **30**, both of which were fully protected by benzoyl and/or benzyl groups. Since the β-face of the Gal ring for both acceptors (**23** and **3**) is partially

occupied by 4,6-*O*-benzylidene or 6-*O*-benzoyl, respectively, it should be much easier for the bulky donor to attack from the α-face (2-substitution). However, for small electrophiles like benzyl, the influence from the steric effect of the donor is not so pronounced, and consequently the substitution takes place predominantly at the more reactive 3-OH.

To solve this problem of regioselectivity, one can either decrease the size of the donor by changing the protecting groups, such as acetyls, or protecting the acceptor at both the 2- and 6-position to prevent the side reaction. Here, we took the second option and prepared the 2,6-*O*-disubstituted galactosyl acceptor.

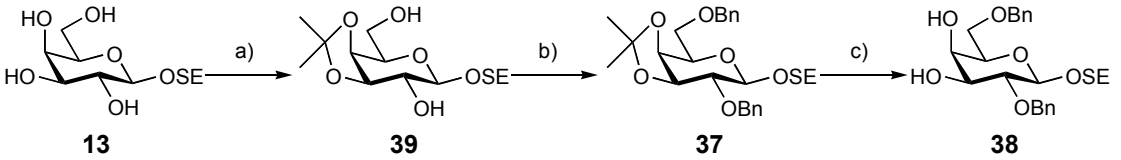


3 **35** **36** **37**

Scheme 9: a) aceton, $TsOH \cdot H_2O$, reflux, 1 h (82%); b) benzyl bromide, NaH, DMF, rt, 16 h.

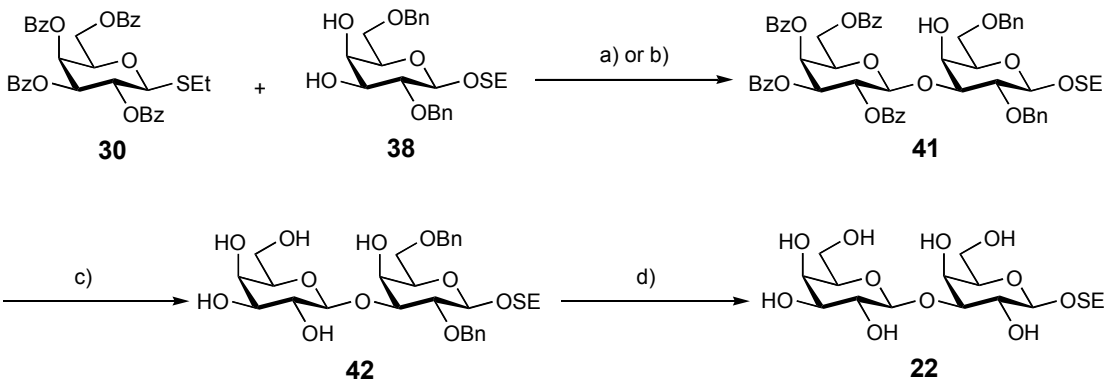
As shown in *scheme* 9, the 6-*O*-benzoylated Gal derivative **3** was treated with acetone in the presence of $TsOH \cdot H_2O$, generating the 3,4-*O*-isopropylidene derivative **35** in 82% yield. Subsequent benzylation at the 2-position was carried out with benzyl bromide and NaH in DMF at rt. It took 16h for the starting material to be completely consumed as observed by TLC. The product, although appears as one single spot on TLC, is a mixture of expected product **36** and byproduct **37** with a ratio of 1:2.4 in favor of **37**. A trace of water included in the reagent and/or the solvent might convert NaH to NaOH, which in turn hydrolyzed the 6-*O*-benzoyl, followed by benzylation at the free 6-OH.

Instead of repeating the former procedure, we adopted another strategy to prepare the acceptor **38** within fewer steps as shown in *scheme* 10. Starting from the known building block **13**, 3,4-*O*-isopropylidene derivative **39** was obtained in 85% yield by treatment with 2,2-dimethoxypropane and *cat*. $TsOH \cdot H_2O$ in DMF. After protecting the 2- and the 6-OH as benzyl ethers, the isopropylidene was cleaved under acidic condition to afford the 2,6-diprotected galactosyl acceptor **38** in 65% overall yield (**13→38**).



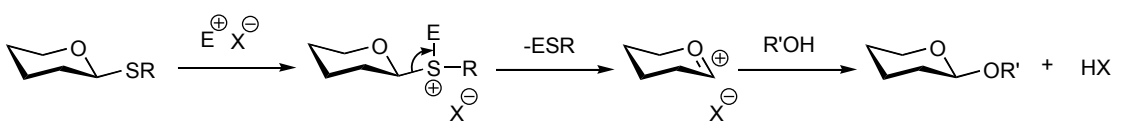
Scheme 10: a) 2,2-dimethoxypropane, $TsOH \cdot H_2O$, DMF, 80°C, 3 h (85%); b) benzyl bromide, NaH, DMF, 0°C, 1.5 h (88%); c) 80% aq. AcOH, 90°C, 1 h (92%).

With the properly protected acceptor in hand, the coupling reaction with donor **30** was carried out under two reaction conditions: NIS-TfOH or dimethyl(methylthio)sulfonium triflate (DMTST, **40**)[147] (*scheme* 11), affording Galβ(1→3)Gal derivative **41** in 60% and 87% yields, respectively.



Scheme 11: a) NIS, TfOH, CH_2Cl_2, -30°C, 1 h (60%); b) DMTST, CH_2Cl_2, 7°C, 14 h (87%); c) NaOMe, MeOH, rt, 2 h (85%); d) H_2, 10% Pd-C, MeOH, rt, 3 h (83%).

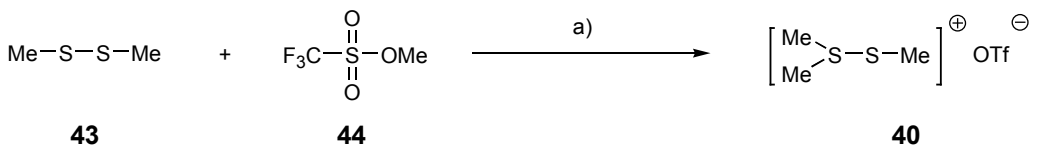
Both promoters play a similar role in glycosylation reactions with thioglycoside donors as shown in *scheme* 12. The soft electrophile E^+ (e.g. I^+ from NIS, and MeS^+ from DMTST) attaches to the sulfur functionality of the glycosyl donor, whereafter the anomeric carbo-sulfur bond is broken to generate an oxycarbenium ion (with counterion X^-, *e.g.* TfO^- in both cases), which then reacts with the acceptor nucleophile to yield the *O*-glycoside.[148]



Scheme 12: General mechanism of glycosylation with thioglycoside donors.

In the case of this glycosylation reaction, although donor **30** is deactivated by the 4 electron-withdrawing benzoyl groups, 3-OH of the acceptor **38** is highly nucleophilic

due to the electron-donating effect from 2,6-*O*-dibenzyl substitution. Therefore, it is reasonable that higher yield was achieved with DMTST, a promoter with modest reactivity that can match perfectly with the reactivity of the donor/acceptor couple, as compared with the one with more reactive NIS-TfOH promoter. It is notable that DMTST, which was prepared from methyl disulfide (**43**) and methyl trifluoromethanesulfonate (MeOTf, **44**) in CH_2Cl_2 (*scheme* 13) is extremely sensitive to H_2O, O_2, light, and heat. Therefore, great cautions should be taken during its preparation, utilization, and storage.



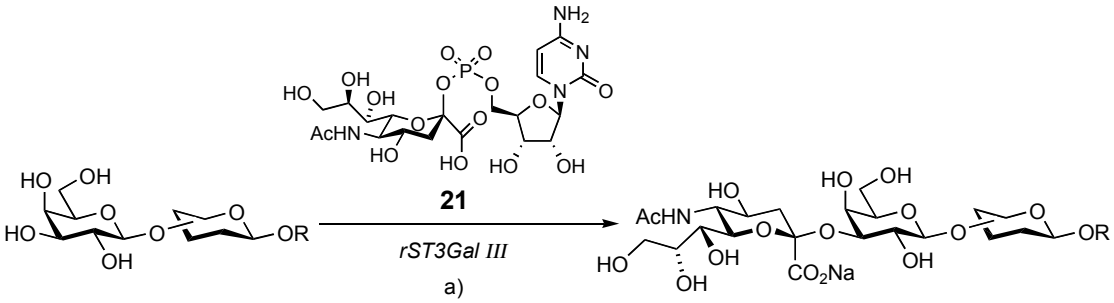
43 **44** **40**

Scheme 13: a) CH_2Cl_2, rt, 16 h (87%).

After smooth removal of the benzoate and benzyl protecting groups by transesterification and hydrogenation successively, Galβ(1→3)Galβ-OSE (**22**) was obtained in 72% yield (**41→42→22**).

1.2.2. Enzymatic sialylation

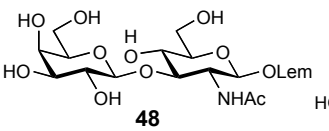
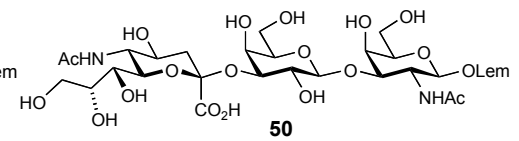
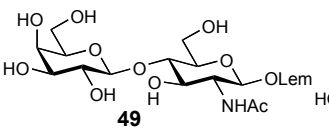
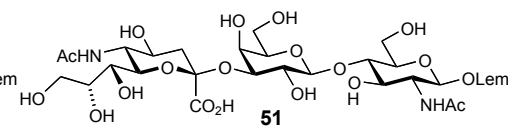
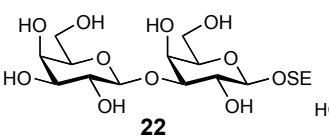
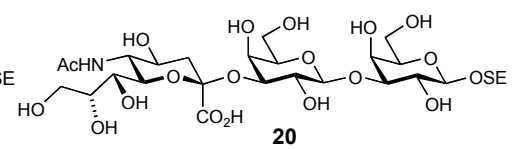
As discussed in Introduction part (**3.2.**), the enzymatic oligosaccharide synthesis employing sialyltransferases as biocatalysts offers an alternative approach with excellent stereoselectivity and generally good to excellent yield.[118,119,149,150] Furthermore, the synthetic value of these enzymes would be greatly enhanced if they would tolerate a broad spectrum of acceptor molecules in addition to their specificity in *vivo*.[120] Previous studies indicated that the natural substrates for *ST3Gal III* are type I [Galβ(1→3)Glc*N*Ac] and type II [Galβ(1→4)Glc*N*Ac] disaccharides.[122-124] In addition, it was shown that *ST3Gal III* accepts a wide variety of substituents at the glucosamine nitrogen,[120,151] as well as lactal, lactose and 2-*O*-pivaloyllactose.[119] Here, *rST3Gal III* (EC 2.4.99.6) was used to transfer the sialic acid moiety from CMP-Neu5Ac (**21**) to the 3-OH of the terminal galactose of non-natural substrates as shown in *scheme* 14.

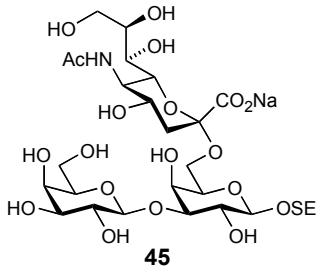
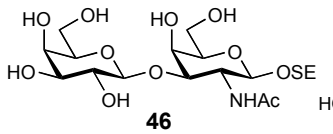
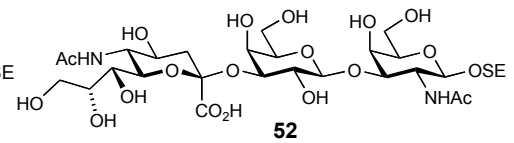
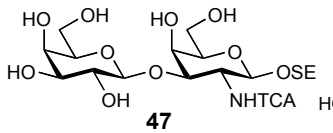
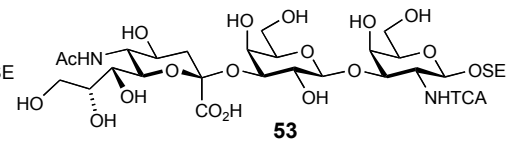


Scheme 14: Enzymatic sialylation with *rST3Gal III*, a) pH 6.5, BSA, CIAP, sodium cacodylate buffer, $MnCl_2$.

In addition to the chemically synthesized disaccharide Galβ(1→3)Galβ-OSE (**22**), trisaccharide Galβ(1→3)[Neu5Acα(2→6)]Galβ-OSE (**45**) (synthesis will be discussed in **1.4.**), Galβ(1→3)Gal*N*Acβ-OSE (**46**) and Galβ(1→3)Gal*N*TCAβ-OSE (**47**),[152] together with natural substrates type I **48**[153,154] and type II **49**[154,155] were incubated with CMP-Neu5Ac and *rST3Gal III* (9 U/L) following our standard sialylation protocol.[156] After 17-24 hours of incubation, one additional aliquot of transferase was added (except for the natural substrates **48** and **49**). However, the addition of a further aliquot of *rST3Gal III* and CMP-Neu5Ac did not further increase the yields anymore (*table* 4).

Table 4: Isolated yields and kinetic data of the enzymatic sialylations.[a)]

Entry	Acceptor[b)]	Product	Isolated product [%][c)]	Recovered acceptor [%]	V_{max} $\left[\frac{\text{nmol}}{\text{mL}\cdot\text{min}}\right]$	K_m [μM]
1	 **48**	 **50**	90	-	3.42	66.08
2	 **49**	 **51**	76	-	3.19	87.61
3	 **22**	 **20**	59	40	0.43	990.51

4	 **45**	no reaction	-	95	-	-
5[d)]	 **46**	 **52**	56	44	1.11	995.55
6[d)]	 **47**	 **53**	36	62	0.89	811.64

a). substrates and CMP-Neu5Ac were incubated with *rST3Gal III* for 3-5 days at 37 °C in a mixture of 50 mM sodium cacodylate buffer (pH 6.5), 60 mM $MnCl_2$-solution and water containing BSA and CIAP (EC 3.1.3.1); b). **48**, **49**: 3 mg, **21**, **45**, **46**, **47**: ~10 mg; c). the course of the reactions was monitored by TLC (silica gel, DCM/MeOH/H_2O 10:4:0.8); d). prepared by Dr. Oliver Schwardt, Institute of Molecular Pharmacy, University of Basel.

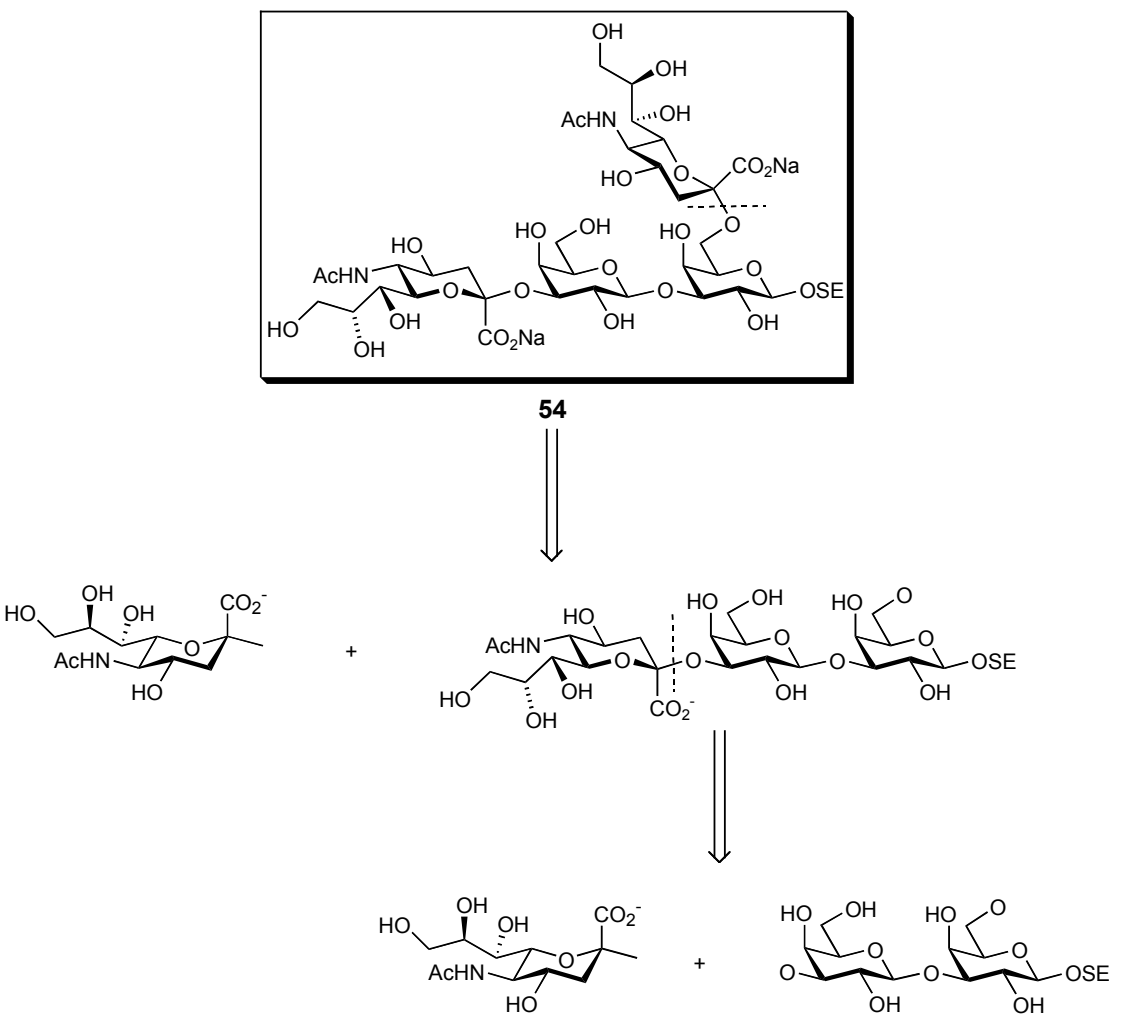
The natural substrates **48** and **49** were converted quantitatively into the corresponding trisaccharides **50** and **51** (*table* 4, *entries 1 and 2*). In addition, the disaccharides **22**, **46** and **47** were also sialylated by *rST3Gal III* affording the corresponding trisaccharides **20**, **52** and **53** in acceptable yields (*entries 3, 5 and 6*). In all three cases, the unreacted substrates could be recovered almost quantitively. The kinetic data for the sialylations indicate that the activity of *rST3Gal III* towards the substrates **22**, **46** and **47** is reduced about 10-fold compared to the one for its natural substrates **48** and **59**. As expected, the transfer efficiency V_{max}/K_m is much lower for the unnatural substrates. This explains the incomplete, but still preparatively useful conversion of the type III disaccharides. Probably, due to the bulkiness of the substituent in the 6-position of galactose, the 6-*O*-sialylated trisaccharide **45** was not tolerated as substrate by *rST3Gal III (entry 4)*.[152,157]

The introduction of a sialic acid unit was indicated by signals in ^{13}C NMR at approximately 100 ppm and 40 ppm, which are characteristic for the C-2 and C-3 of an α-linked sialic acid.[120,151,155] In addition, down-field shift (~4 ppm) of the galactose C-3 in ^{13}C NMR and approximately 0.6 ppm of the galactose H-3 in ^{1}H NMR

confirmed the regioselective introduction of sialic acid in the 3-position of the galactose moiety.[122-124]

1.3. Synthesis of Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)]Galβ-OSE (54)

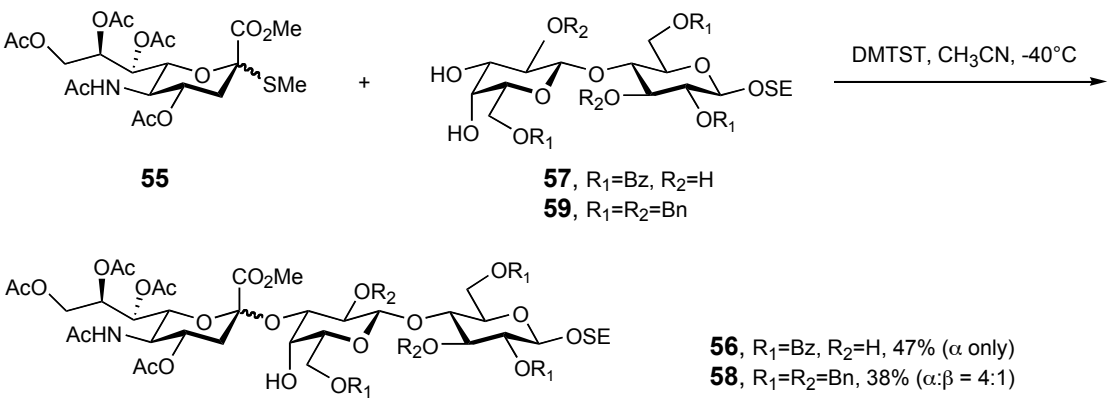
"Chol-1" gangliosides, such as GQ1bα, GT1aα that contain an additional α2,6-linked sialic acid on Gal*N*Ac display enhanced potency for MAG binding, indicating the important role this moiety plays in the binding event.[75] Based on this observation, tetrasaccharide Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)]Galβ-OSE (**54**) was also planned to synthesize. Since the unavailability of α(2→6)-sialyltransferase, different strategy was employed for chemical synthesis of **54**. According to the retro-synthesis (*scheme* 15), a properly protected disaccharide and one sialyl donor were needed to carry out the whole synthesis.



Scheme 15: Retro-synthesis of Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)]Galβ-OSE (**54**).

1.3.1. Preparation of the disaccharide core

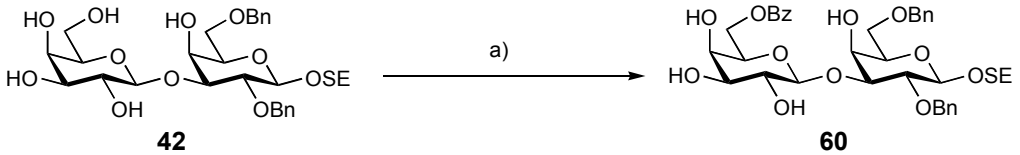
As indicated in *scheme* 15, Galβ(1→3)Gal disaccharide core should be protected in such a way that the first sialic acid moiety can be introduced to the 3-position of the terminal Gal, and in turn, the second one goes to the 6-position of Gal at the reducing end. Concerning the α2,3-sialylation, it is found that good yields and high α-anomeric selectivities were achieved when glycosyl acceptors were partially protected (glycosylation of diol and triols).[121,137,138] When the acceptor is a galactose derivative, the best results were obtained by the use of 2,3,4-trihydroxy derivatives rather than the glycosylation of similar 3,4-diols. As shown in *scheme* 16, with the same sialyl donor **55** and reaction system (DMTST, CH_3CN, -40°C), 47% yield of pure α-anomer **56** was achieved with a lactoside derivative containing 2,3,4-trihydroxy in the galactose moiety **57**. However, yield and stereoselectivity were much lower (**58**, 38%, α:β=4:1) when 2-OH was protected as a benzyl ether **59**.[121,158]



Scheme 16: Sialylations of diol and triol galactosyl acceptors.

The regioselectivity of sialylation is due to the greater reactivity of the equatorial OHs (2-OH and 3-OH) compared to the axial one (4-OH). Furthermore, the 2-OH has a lower nucleophilicity due to the electron-withdrawing effect of the adjacent anomeric center. When a similar sialylation was performed with a galactosyl acceptor having only a free 3-OH, both the yield and anomeric stereoselectivity were significantly reduced.[121] Additionally, it should also be considered that procedures for the preparation of glycosyl acceptors having several free hydroxyls are often easier to conduct and, hence, may offer shorter routes to oligosaccharides. Based on these studies, compound **60** was selected as the disaccharide core for further α2,3- and

α2,6-sialylation. As show in *scheme* 17, it can be reached in one step from the known building block **42**.



Scheme 17: a) benzoyl cyanide (0.9 equiv.), CH_3CN-Et_3N (4 : 1), -40~-45°C, 1.5 h (80%).

Table 5: Studies of selective 6-*O* benzoylation conditions of **42**

Entry	Benzoyl cyanide[a)]	CH_3CN-Et_3N	Temp.	Time	**60**[b)]
1[c)]	1.2 equiv.	4 : 1	-30°C	2 h	42%
2[d)]	1.1 equiv.	4 : 1	-40~-45°C	2 h	65%
3[d)]	1.1 equiv.	4 : 1[e)]	-80°C	3 h	[f)]
4[d)]	1.1 equiv.	[g)]	-40~-45°C	2 h	[f)]
5[d)]	0.9 equiv.	4 : 1	-40~-45°C	1.5 h	80%[h)]

a). equivalent to **42**; b). separated yields of **60**; c). benzoyl cyanide was added in one portion to **42** in solvents; d). benzoyl cyanide was dissolved in CH_3CN (volumn depends on the reaction scale, see experimental part) and added by drops; e). CH_2Cl_2 was used instead of CH_3CN because of its low melting point; f). no separated yields, reactions were followed by TLC, from which product and unreacted starting material, together with multi-benzoylated byproducts were detected; g). 1.2 equivalent of Et_3N to **42** was used; h). calculated according to the amount of benzoyl cyanide.

As summarized in *table* 5, we first tried the selective 6-*O*-benzoylation with the standard procedure employed before (*entry 1*), affording the product **60** in 42% yield, which was largely due to the side reactions of multiple benzoylation. Although the multi-benzoylated byproducts can be separated from the expected product, and recovered to be **42** after Zemplén deprotection, the low yield prompted us to modify the reaction conditions for higher selectivity.

In contrast to the standard procedure, the way of adding benzoyl cyanide was modified: it was dissolved in CH_3CN and added dropwise in order to maintain its low concentration in the reaction mixture, thus prevent side reactions to take place. In addition, temperature effects were also studied. When the reaction took place at -40~-

45°C, combined with the effect of slow adding of benzoyl cyanide, the yield rose to 65% (*entry 2*). However, no improvement was observed by further decreasing the temperature to –80°C (*entry 3*). The role of base in the reaction was studied by minimizing the amount of Et_3N to 1.2 equiv., which showed no better result (*entry 4*). Furthermore, the amount of benzoyl cyanide was decreased to 0.9 equiv., which gave the highest yield of 80% (*entry 5*). Therefore, the best reaction conditions were generated: 0.9 equiv. of benzoyl cyanide dissolved in CH_3CN was added dropwise to the starting material **42** in CH_3CN-Et_3N (4:1) at -40~-45°C, followed by reacting for 1.5 h at the same temperature. Thus, properly protected disaccharide core **60** was achieved in 80% yield.

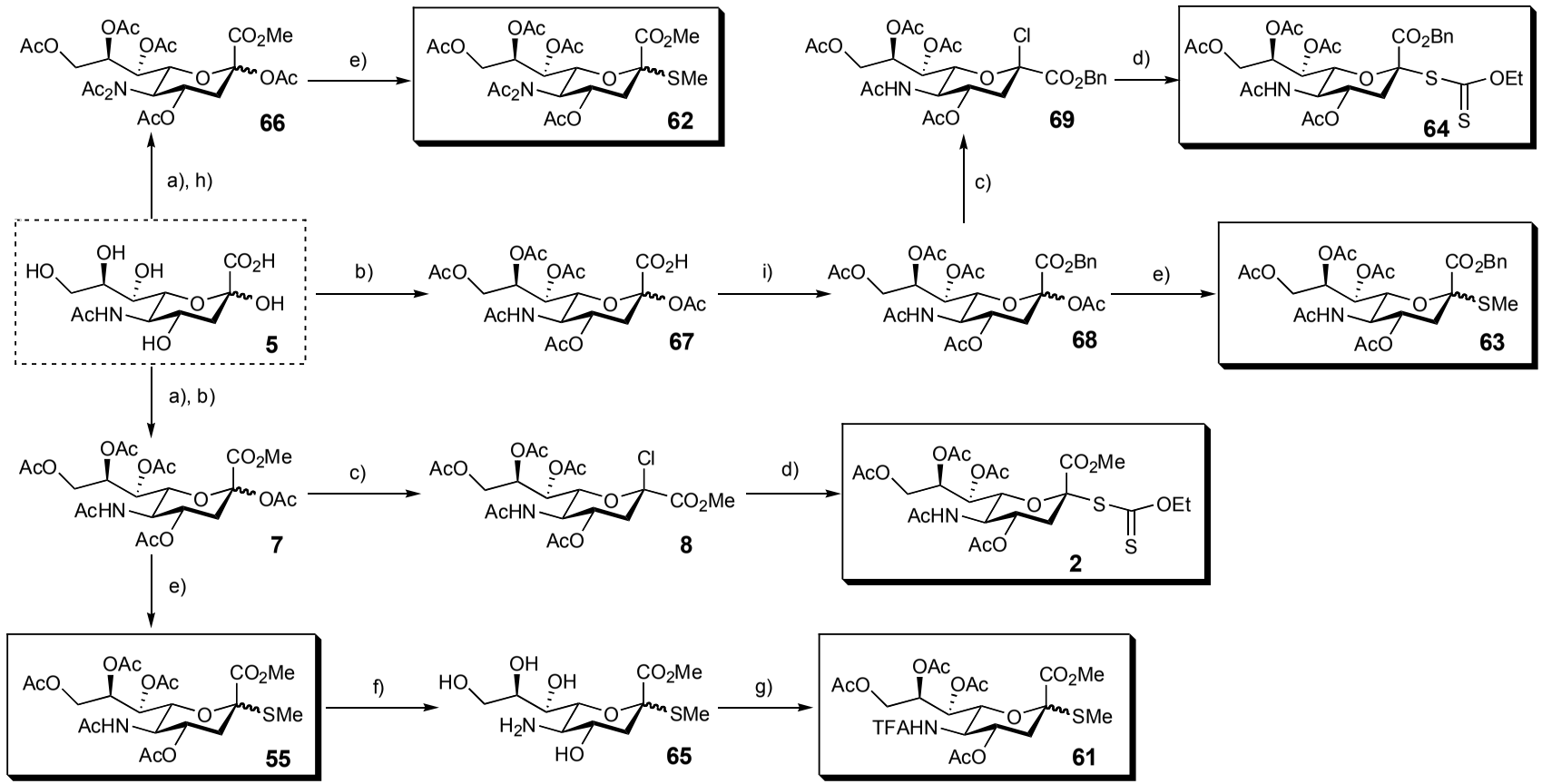
1.3.2. Studies of α2,3-sialylations

Sialylation with high yield and stereoselectivity is one of the most challenging aspects in oligosaccharide chemistry, which is mostly due to the following reasons:

1) The tertiary anomeric center is not only sterically hindered, but the reactivity is also electronically disfavored by the carboxylic acid moiety;
2) No neighboring group is present at C-3 to guide the nucleophilic attack by the glycosyl acceptor, additionally, the deoxy moiety in combination with the electron-withdrawing carboxylic acid leads to β-elimination, the most serious side reaction which forms sialic acid glycal;
3) The *N*-acetyl group may react with strong electrophiles to give byproducts;
4) The β-sialoside is the thermodynamically favored product, while virtually all sialic acid containing saccharides in nature carry the sialic acid moiety in α-configuration.[132,159]

To address these difficulties, various explorations were carried out concerning *e.g.* functions of the activating group at the anomeric center, the participation of the C-3 auxiliary, the solvent effect of CH_3CN, the substituent effect of carboxylic ester and so on, which achieved considerable improvements during the past years.[132,159]

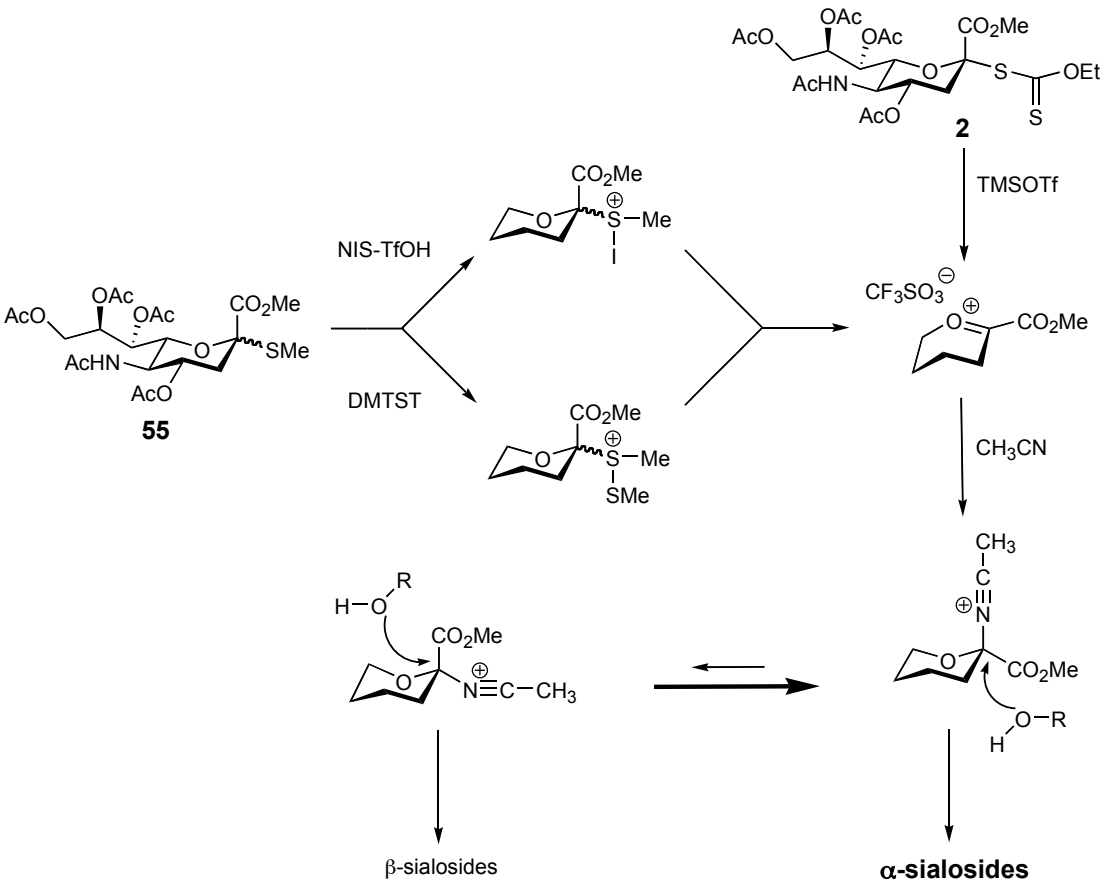
In sialylation reactions, the character of the donor is a major determinant of the anomeric selectivity. It depends on the leaving group at the anomeric center, the protecting group of amine at C-5 and the ester of the carboxylic acid. It should be noticed that the most successful sialyl donors use rather unusual anomeric leaving groups.[121] For example, fluorides and trichloroacetimidates are most widely applied for common glycosyl donors, whereas sialyl donors rarely or never possess these leaving groups. Among the common activating groups of sialyl donors, the family of 2-thio derivatives is one of the most attractive sialyl donors, such as 2-thioalkyl/aryl and 2-xantho sialyl derivatives.[148] Due to the excellent chemical stability, thio groups offer efficient protection of the anomeric center and are compatible with many reaction conditions often employed in carbohydrate chemistry. However, in the presence of soft electrophiles (NIS-TfOH, DMTST, *etc*.), thioglycosides can be activated and used directly in glycosylations. Another attractive feature is that they can be transformed into a range of other glycosyl donors. In view of the side reaction caused by the *N*-acetyl group, modifications were carried out by employing more electron-withdrawing groups, like *N*-acetylacetamido (*N*-Ac_2),[160] *N*-trichloroacetamido (*N*-TFA),[161] or masking it by N_3,[162] therefore decreased the nucleophilicity, in turn avoided its reaction with electrophile and resulted in improved yields and stereoselectivities. Recently, the auxiliary role of esters of the carboxylic acid either by "long range participation", forming β-intermediate which facilitate α-glycosidation,[163,164] or by enhancing the solvent effect by stabilizing the β-oriented nitrilium ion[165] has been explored.



Scheme 18: a) *cat.* Amberlyst 15, MeOH, rt, 16 h (93%); b) Ac_2O, py, DMAP, rt, 48 h (**7**, 91%, **67** quant.); c) AcCl, *conc.* HCl, CH_2Cl_2, rt, 16 h (**8**, 77%; **69**, 95%); d) potassium *O*-ethyl dithiocarbonate, TBAHS, 5% aq. $NaHCO_3$, AcOEt, rt, 3 h (**2**, 51%; **64**, 90%); **e**) TMSSMe, TMSOTf, CH_2Cl_2, 45°C, 5 h, rt, 16h (**55**, 92%; **62**, 65%; **63**, 82%); f) MsOH, MeOH, 60°C, 24 h (100%); g) i. CF_3COOMe, Et_3N, MeOH, 2 h, ii. Ac_2O, py, rt, 5 h (55%, **55→61**); h) IPA, $TsOH·H_2O$, 65°C, 16 h (80%); i) KF, BnBr, DMF, rt, 20 h (89%).

Here, through the combination of 2-thiomethyl or 2-xanthate as the leaving groups, *N*-Ac, *N*-Ac_2, *N*-TFA at C-5 and methyl or benzyl carboxylate, the six sialyl donors **2**, **55**, **61**-**64** were readily prepared according to *scheme* 18. Starting from Neu5Ac (**5**), methylation and acetylation gave fully protected **7**, which can either be converted to 2-xantho sialoside methyl ester **2** (see also *scheme* 2), or coupled with (methylthio)trimethylsilane (TMSSMe) and *cat.* TMSOTf giving 2-thiomethyl sialyl donor **55** in a quantitative yield as a mixture of α/β-anomers.[166] It should be noted that in sialylations, the two anomers have very comparable glycosyl donor properties and therefore do not need to be separated.[121] Removal of *O*-acetyls and *N*-acetyl was accomplished quantitatively by treating **55** with methylsulfonic acid (MsOH) in MeOH at 60°C.[167] Subsequent selective *N*-trifluoroacetylation of **65** with methyl trifluoroacetate in the presence of Et_3N in MeOH followed by *O*-acetylation with Ac_2O in py gave sialyl donor **61** in overall yield of 55% (**55→61**).[161] By employing *iso*-propenyl acetate (IPA) as an acetylating reagent in the presence of *cat.* $TsOH{\cdot}H_2O$, sialyl derivative **66** with *N*-diacetyl functionality can be obtained in 80% yield from Neu5Ac methyl ester.[160] With the above-described procedure of thiomethylation, **62** was readily obtained in 65% yield. Meanwhile, Neu5Ac benzyl ester **67** was prepared by acetylation of **5** (**→67** quant.), and following benzylation with BnBr in the presence of KF in DMF (**→68**, 89%).[168] The resulting **68** can either be transformed to 2-thiomethyl sialyl donor **63** in 82% yield, or quantitatively converted to 2-xantho sialoside benzyl ester **64** through 2-chloro sialyl derivative **69**.[169]

With these six sialyl donors in hand, sialylations with disaccharide **60** or monosaccharide **3** were carried out with NIS-TfOH or DMTST as promoters, in CH_3CN at low temperature. The proposed mechanism for α-predominant sialylation with 2-thio sialyl donors in CH_3CN is show in *scheme* 19.[159]

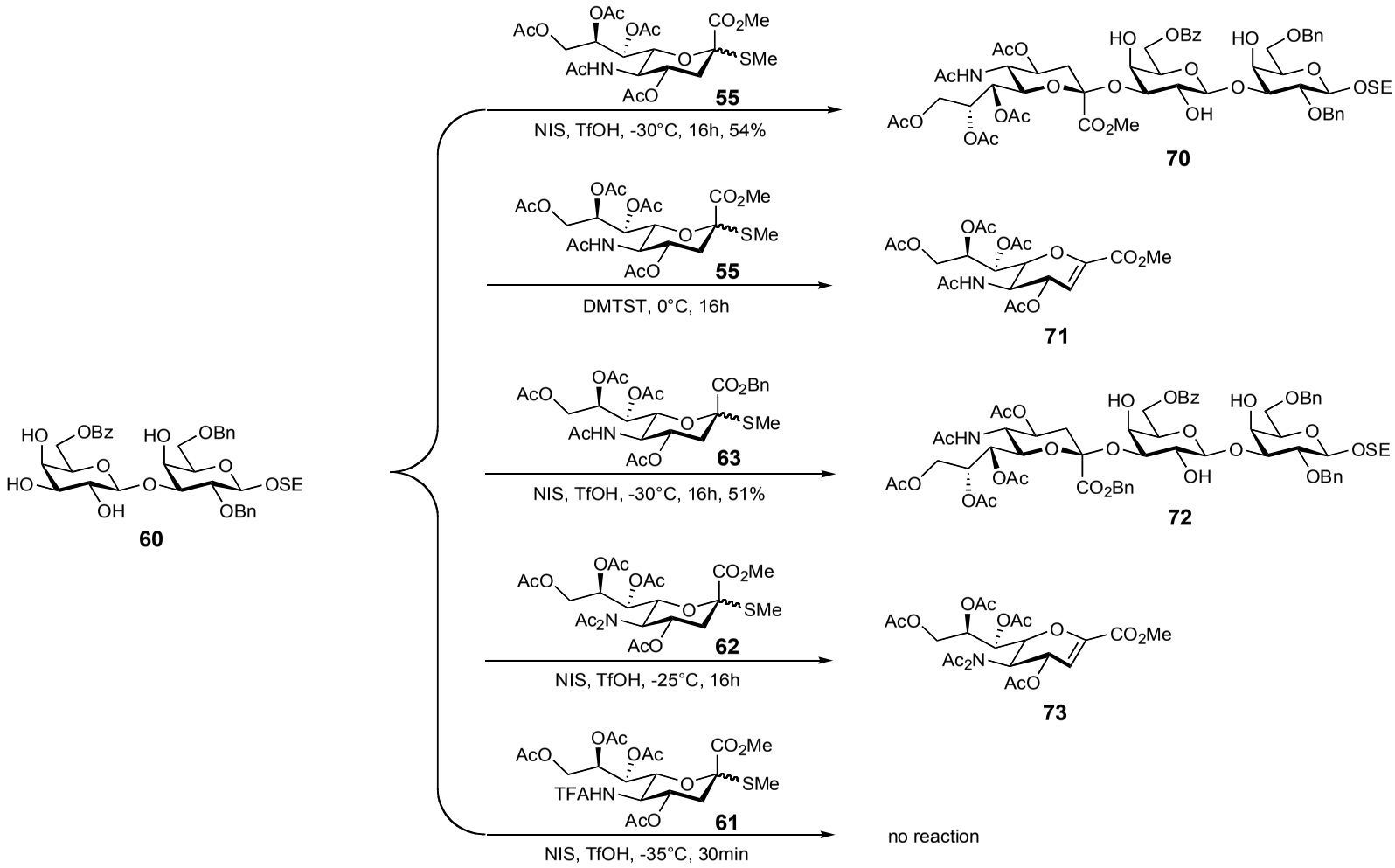


Scheme 19: Proposed mechanism for α-predominant sialylations with 2-thio sialyl donors in CH_3CN.

The soft electrophilic species (I^+, MeS^+) formed under the reaction conditions react with the lone pair of sulfur resulting in the formation of a sulfonium intermediate which is an excellent leaving group. Upon leaving, the generated oxycarbenium ion can be attacked by nitrogen of CH_3CN to give a nitrilium ion, which adopts a preferred axial (β) configuration. Subsequent nucleophilic substitution of the nitrilium ion with the acceptor gives predominantly the α-sialosides.

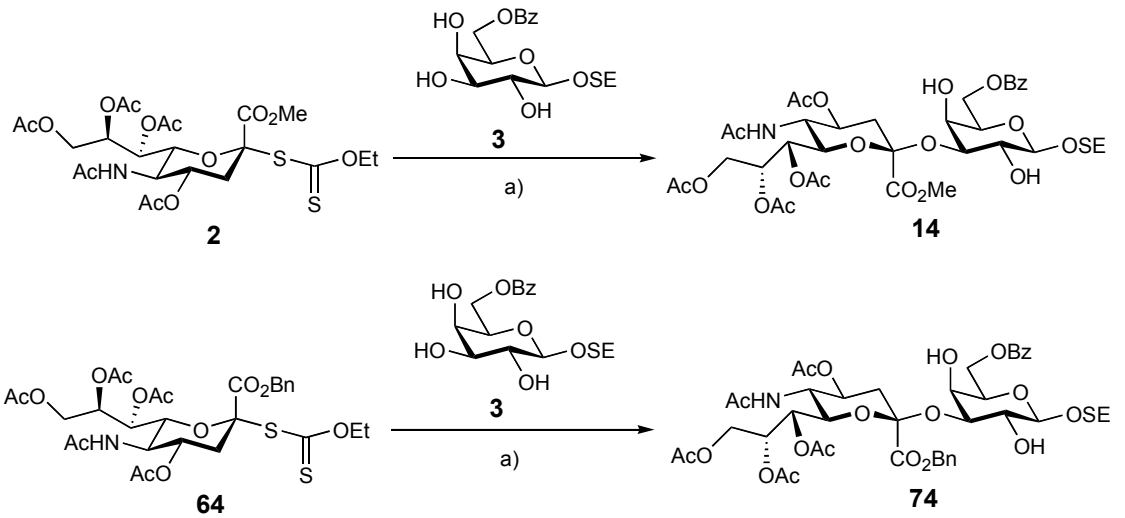
As shown in *scheme* 20, 2-thiomethyl sialyl donors **55**, **61**-**63** were coupled with disaccharide **60** using NIS-TfOH or DMTST as promoters in CH_3CN at low temperature. The sialylation with **55**, the most commonly used sialyl donor, achieved the highest yield of α-sialoside **70** (54%) by employing NIS-TfOH as the promoter in CH_3CN at –35°C overnight. However, only the sialic acid glycal **71** (39%) together with quantitatively recovered starting material **60** were obtained when DMTST was used in CH_3CN at 0°C overnight, which is the general procedure with DMTST. The significant difference between the two reactions is the reaction temperature. Due to

the low reactivity of DMTST compared to NIS-TfOH, the reaction was carried out at 0°C (-35°C with NIS-TfOH), where the elimination to the corresponding glycal become thermodynamically favored compared to the sialylation. A similar high yield (**72**, 51%) was achieved with 2-thiomethyl sialoside benzyl ester **63** under the same condition, indicating methyl and benzyl esters have similar influence on this sialylation. Surprisingly, in contrast to the literatures,[160,161] no major product was observed by using **61** as sialyl donor, and only 34% of glycal **73** was obtained with donor **62**. Thus, 2-thiomethyl sialyl donors with both methyl and benzyl esters showed comparable results, whereas *N*-acetyl at C-5 showed to be superior compared to *N*-diacetyl and *N*-trifluoroacetyl functionalities.



Scheme 20: Sialylations of **60** with various 2-thiomethyl sialyl donors **55**, **61**-**63** under the promotion of NIS-TfOH or DMTST.

To further elucidate the function of the leaving groups at the reducing end, donor **2** and **64** were reacted with acceptor **3** as shown in *scheme* 21. By coupling of **2** and **3**, disaccharide **14** was obtained in 29% yield (see also *scheme* 4). The same reaction conditions were employed with sialyl donor **64**, resulting in product **74** with comparable yield (26%).



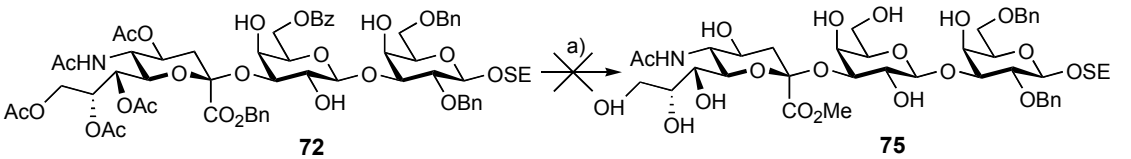
Scheme 21. Sialylations of **3** with 2-xantho sialyl donors: a) NIS, TfOH, CH_2Cl_2-CH_3CN(2:3), -70°C, 16h (**14** 29%, **74** 26%).

Based on these studies, sialyl donors **55**, **63** with thiomethyl group at the anomeric center, *N*-acetyl at C-5 and methyl or benzyl ester of the carboxylic acid showed the best reactivity and α-stereoselectivity. They were therefore employed in α2,3- and α2,6- sialylations in this work.

1.3.3. α2,6-sialylation

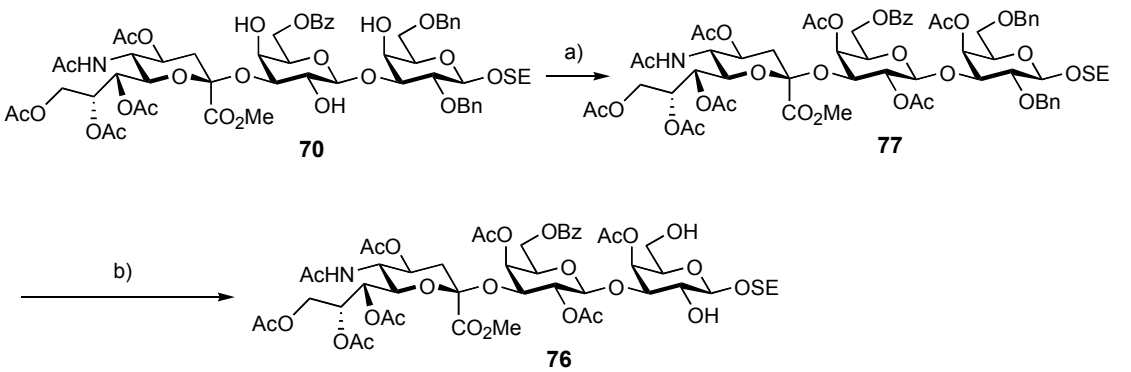
Trisaccharides **70** and **72** served as acceptors for subsequent α2,6-sialylation after manipulations. In the case of **72**, the carboxylic acid of sialic acid moiety and the 2,6-positions in galactose at the reducing end are protected with benzyl groups. Before the cleavage of 2,6-*O*-dibenzyl to generate free 6-OH for further glycosylation, benzyl carboxylate had to be converted to the corresponding methyl ester by transesterification with NaOMe in MeOH (*scheme* 22). The resulting **75** could be in turn re-protected with acetyls followed by debenzylation. Unfortunately, no signal of methyl ester was observed in ^{1}H NMR spectrum after transesterification. The possible reason might be that hydrolysis took place to generate the free carboxylic acid due to

a trace of water included in the reagent or the solvent. However, due to the limited amount of **72** available, we used trisaccharide **70** to continue the synthesis of target tetrasaccharide **54**.



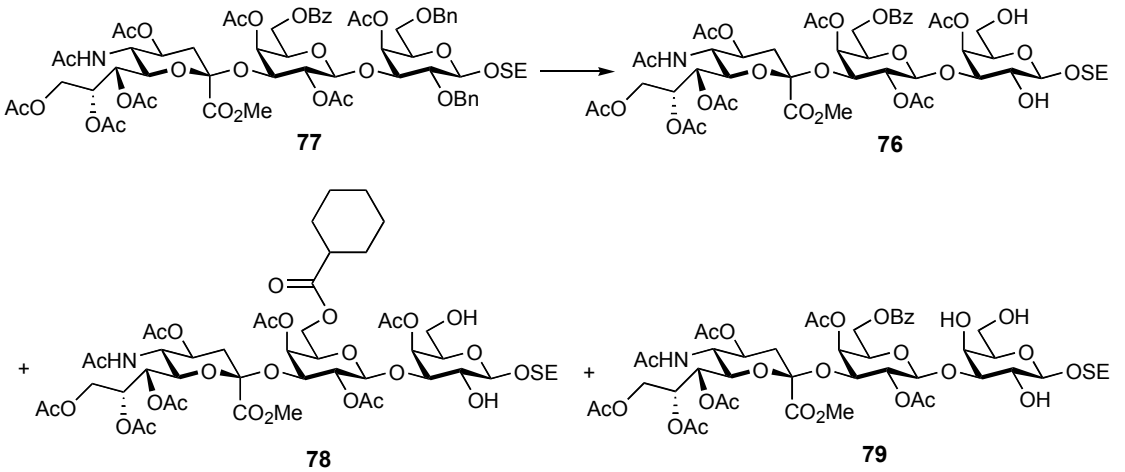
Scheme 22: a) NaOMe, MeOH, rt, 16h (no product).

By two simple steps, acetylation and debenzylation, the trisaccharide acceptor **76** could be obtained. As shown in *scheme* 23, **70** was acetylated with Ac_2O in pyridine giving **77** in 80% yield. Since the debenzylation step by hydrogenation was not straightforward, various reaction conditions were examined as summarized in *table* 6.



Scheme 23: a) Ac_2O, py, rt, 16h (80%); b) 10% pd-C (150% w/w), EtOH-HOAc (5:1), 45°C, 5 h (**76**, 76%; **79**, 15% see *table 3, entry 4*).

Table 6: Studies of debenzylation conditions of **77**



Entry	Catalyst (w/w)	Solvents	Temp.	Time[a)]	Pressure	Yields[b)]
1	10% Pd-C (200%)	dioxane-MeOH (1:4) *cat.* AcOH	rt	68 h	4 bar	50%[c)] **76**:**78**=3:2
2	10% Pd-C (300%)	dioxane-MeOH-AcOH (1:4:1)	rt	3 d	4 bar	70%[c)] **76**:**78**=3:2
3	20% $Pd(OH)_2$ (100%)	EtOH-AcOH (10:1)	45°C	4 d	1 bar	**76**:42%, **79**:30%
4	10% Pd-C (150%)	EtOH-AcOH (5:1)	45°C	5 h	1 bar	**76**:76%, **79**:15%
5	10% Pd-C (150%)	EtOH-AcOH (5:1)	45°C	18 h	1 bar	[d)]

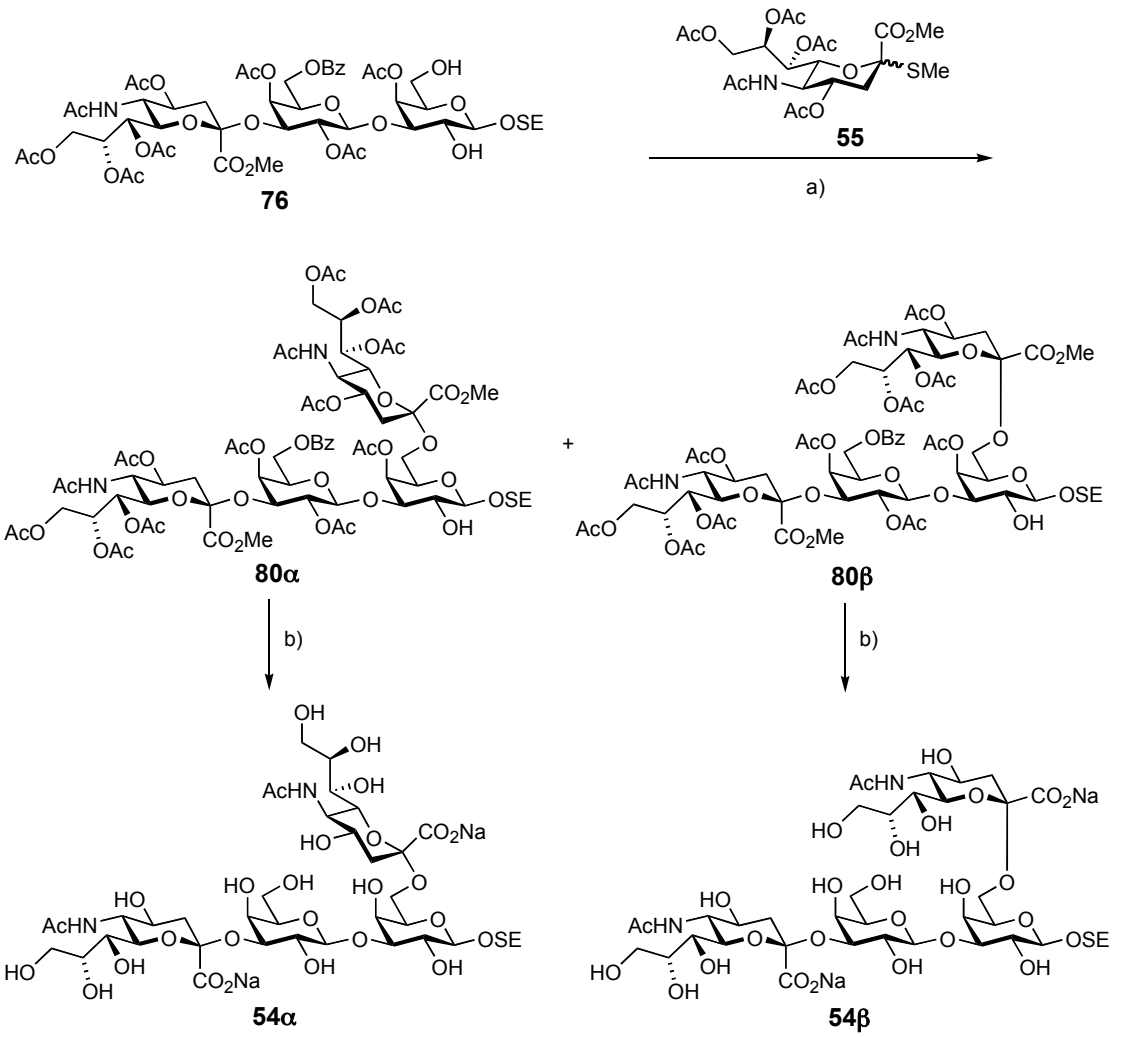
a). followed by TLC, till **77** was completely consumed; b). isolated yields; c). mixture of **76** and **78**, ratios were determined by ^{1}H NMR; d). no isolated yield.

Hydrogenation of **77** under normal conditions (H_2 with *cat*. 10% Pd-C at rt under normal pressure) showed no reaction or to be impractically slow (result not shown in *table* 6). Based on the experience gained from the hydrogenation of **41** (see **1.4.**), more vigorous reaction conditions, *e.g*. large amount of catalyst (10% Pd-C, 200% w/w) and increased pressure (4 bar) were employed, allowing the complete consumption of the starting material within 68 h (*entry 1*). According to the MS and ^{1}H NMR, **76** and **78** were formed in a ratio of 3:2 (**76**:**78**). Under the extremely harsh condition of hydrogenation, the phenyl ring of the benzoate at 6-position was partially reduced to cyclohexane. Since these two substances **76** and **78** have exactly the same Rf value with various systems of developing solvents, it was impossible to follow

their formation by TLC. Under the similar conditions (*entry 2*), 70% of a 3:2 mixture of **76** and **78** was obtained. It is known that 20% $Pd(OH)_2$ is used frequently as the catalyst at high temperature for the debenzylation of the similar molecules.[170-172] By treating **76** with 20% $Pd(OH)_2$ (100% w/w) in EtOH-AcOH (10:1) at 45°C (*entry 3*), the reaction took 4 d to complete, leading to the desired product **76** (42%) and **79** (30%). Gratifyingly, by using 10% Pd-C under similar condition, the reaction was much faster. The starting material was completely consumed after 5 h as indicated by TLC (*entry 4*). As a major product, **76** was obtained in 76% yield, while the side product **79** was formed only to the extent of 15%. It should be noticed that the byproduct appeared at the very early stage of the reaction. Instead of having two impurities (**79** and unreacted **77**), it was practically preferred to let the starting material to be fully consumed before stopping the reaction. By prolonging the reaction time to 18 h, the amount of **79** was increased considerably as indicated by TLC (*entry 5*). As a summary, 2,6-*O*-dibenzyl derivative of galactose at the reducing end is difficult to be selectively removed, possibly due to the steric hindrance that is very critical to heterogeneous hydrogenation. Difficulties of slow and incomplete reaction were encountered under general conditions. By using the harsh conditions, byproducts could not be avoided. Interestingly, different byproducts were obtained according to different conditions employed. By catalytic hydrogenation under high pressure (4 bar), the phenyl ring at the 6-position was reduced to the cyclohexane (*entries 1 and 2*), while at high temperature, axial-*O*-acetyl group at 4-position was cleaved possibly through the alcoholysis under acidic condition (*entries 3-5*).

Besides the traditional hydrogenation method, other debenzylation conditions were also applied. Light-initiated debenzylation[173,174] performed under UV irradiation (375W lamp) by using *N*-bromosuccinimide (NBS) in the presence of aqueous calcium carbonate is one alternative approach for de-*O*-benzylation of sterically hindered benzyl ethers, which is supposed to involve radical bromination at the benzylic position and subsequent hydrolysis of the resulting α-bromo ether. The system of sodium bromate and sodium dithionite ($NaBrO_3$-$Na_2S_2O_4$) in AcOEt-H_2O is also reported to selectively cleave the benzyl ether with a similar mechanism.[175] Unfortunately, both procedures didn't result in the desired product in our case.

By employing the modified catalytic hydrogenation, trisaccharide acceptor **76** was available for the further α2,6-sialylation. The coupling reaction was carried out under similar reaction condition with the same sialyl donor **55** as shown in *scheme* 24. The desired tetrasaccharide with α-configuration **80α** was achieved in 38% yield, along with 12% of β-anomer **80β**. After final deprotection by transesterification with NaOMe in MeOH and following hydrolysis with LiOH in dioxane-H_2O solution, **54α** and **54β** were obtained in 49% and 68% respectively.

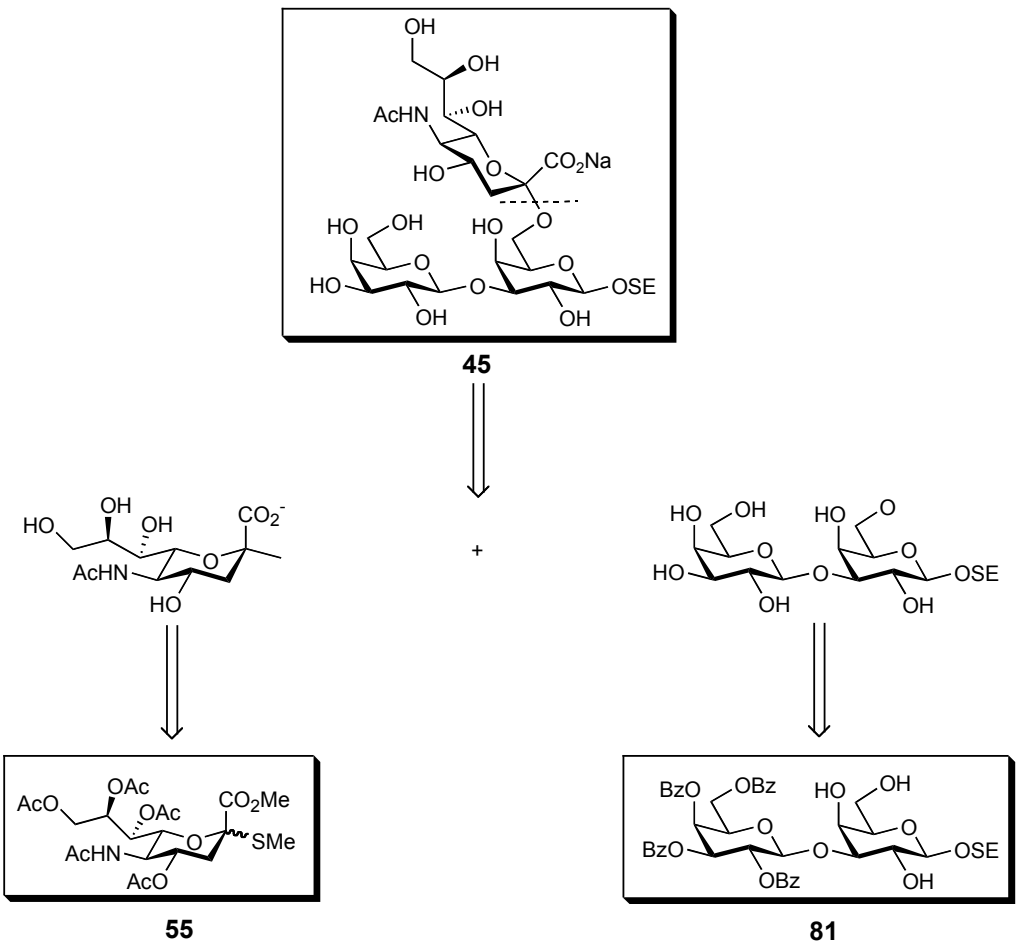


Scheme 24: a) NIS, TfOH, CH_3CN, -40°C, 16 h (**80α**, 38%; **80β**, 12%); b) i. NaOMe, MeOH, rt, 7 h, ii. LiOH (0.1M), dioxane-H_2O (1:1), rt, 16 h, iii. Dowex 50×8 (Na^+) (**54α**, 49%; **54β**, 68%).

1.4. Synthesis of Galβ(1→3)[Neu5Acα(2→6)]Galβ-OSE (45)

It is known from the SAR studies of gangliosides that both sialic acid moieties, the terminal α2,3-linked to Gal and the internal α2,6-linked to Gal*N*Ac are essential for

binding affinity.[56,57] The α2,6-linked sialic acid can enhance the adhesion as shown by "Chol-1" gangaliosides, like GQ1bα, while no activity was found with gangliosides without the terminal α2,3-linked sialic acid moiety, like GM1a.[71] To further elucidate the SAR, trisaccharide **45** was planned to be synthesized. According to the retro-synthesis shown in *scheme* 25, the preparation of **45** was supposed to be straightforward. The sialyl donor **55** was readily available, while the acceptor **81** can be synthesized in one step from building block **41**.

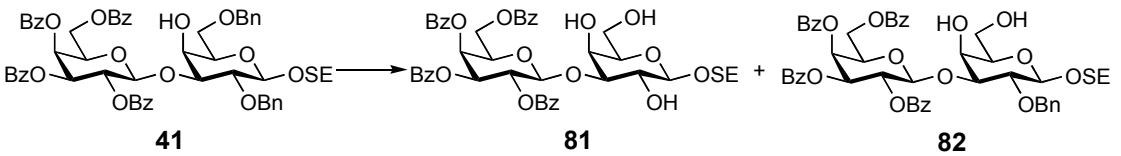


Scheme 25: Retro-synthesis of Galβ(1→3)[Neu5Acα(2→6)]Galβ-OSE (**45**).

Debenzylation of **41** under standard catalytic hydrogenation conditions turned out to be extremely difficult (*table* 7). No reaction was observed under normal conditions (H_2 with *cat*. 10% Pd-C, rt, normal pressure) (*entry 1*). The starting material was completely resistant to the hydrogenation even at high pressure (*entry 2*). Trace of product could be observed by increasing the amount of 10% Pd-C (100% of the amount of **41**), the structure of which was assigned to be **82** (5%), mono-debenzylated

at 6-position (*entry 3*). The desired product **81** was obtained when the amount of Pd-C was further increased to 150% (w/w), adding MeOH and increasing the reaction time as long as 5 d (*entry 4*). Even though, the reaction was not complete as the byproduct **82** and unreacted **41** could be detected from TLC, and the separated yield of **81** was only 28%. The best yield was achieved by raising the amounts of MeOH and AcOH (dioxane-MeOH-AcOH = 1:4:1) and reacting for 9 d at high pressure (*entry 5*, 67%).

Table 7: Studies of debezylation conditions of **41**.



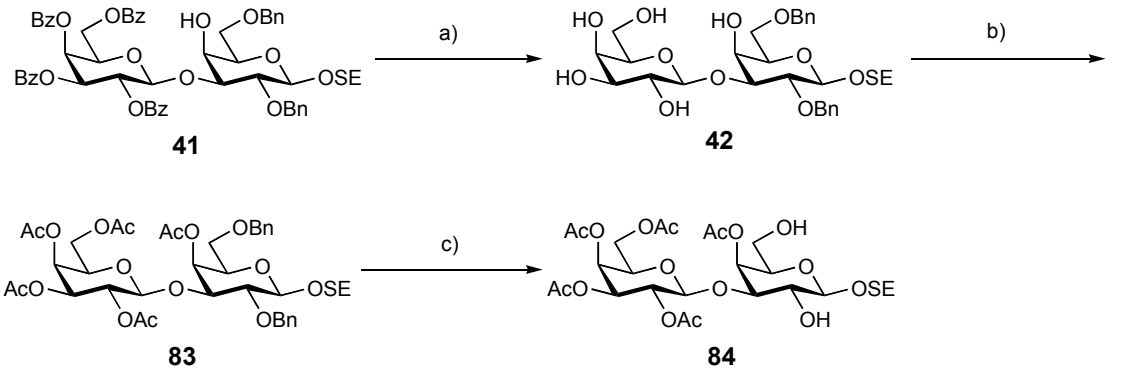
41 **81** **82**

Entry	10% Pd-C (w/w)	Solvents	Temp.	Time	Pressure	**81**[a)]
1	20%	dioxane, *cat*. AcOH	rt	22 h	-	[b)]
2	20%	dioxane, *cat*. AcOH	rt	20 h	4 bar	[b)]
3	100%	dioxane, *cat*. AcOH	rt	15 h	4 bar	[c)]
4	150%	dioxane-MeOH (1:2), *cat*. AcOH	rt	5 d	4 bar	28%
5	150%	dioxane-MeOH-AcOH 1:4:1	rt	9 d	4 bar	67%

a). isolated yields; b). no trace of product could be detected from TLC, **41** was quantatively recovered; c). 5% of **82** and 90% of **41** were obtained after chromatography.

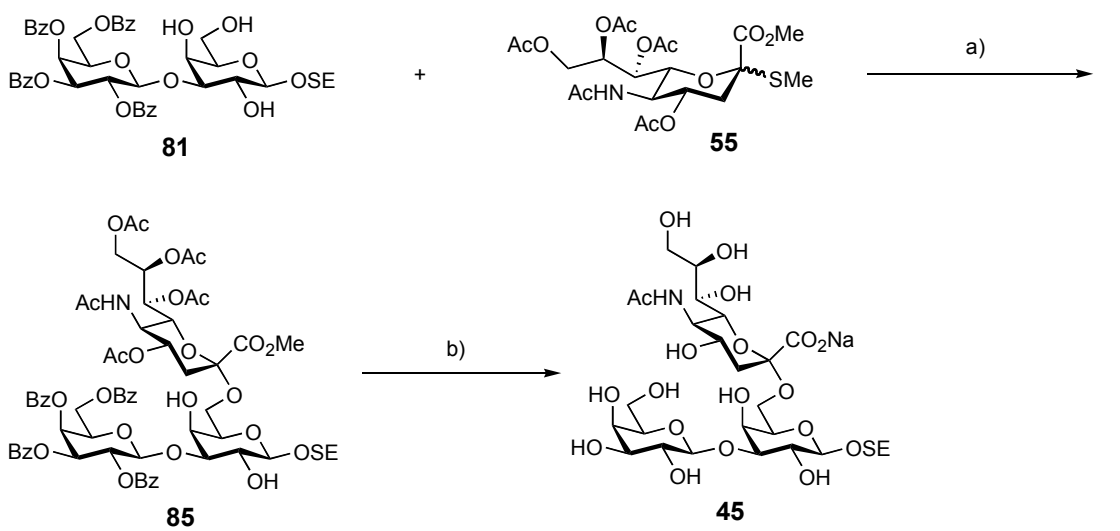
The difficulties encountered in the catalytic hydrogenation of **41** were presumably due to the steric hindrance caused by four benzoyls in the terminal Gal moiety. Although **81** could be obtained in 67%, the harsh condition and the impractically long reaction time prompted further study of this reaction. As shown in *scheme* 26, when the benzoyls were converted to much smaller acetyls in two steps (**41→42→83**), the debenzylated product **84** could be formed quantitatively, which confirmed the

postulated steric influence. It should be noted that the reaction time might be far less than 2 d, since no TLC-control was tried during that period of time.



Scheme 26: a) NaOMe, MeOH, rt, 2 h (87%); b) Ac_2O, py, DMAP, rt, 16 h (92%); c) 10% Pd-C (150% w/w), dioxane-MeOH-AcOH (1:4:1), rt, 4 bar, 2 d (94%).

With the disaccharide **81** in hand, the coupling reaction was carried out under the standard conditions with sialyl donor **55** as shown in *scheme* 27. The desired α-anomer **85** was obtained in 45% yield after chromatography, while the amount of a trace of β-anomer was not determined. The final deprotection by transesterification with NaOMe in MeOH followed by hydrolysis in aq. NaOH afforded **45** in 90% yield.



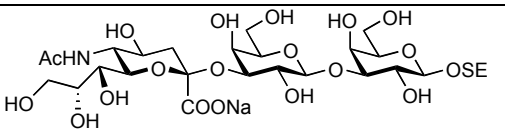
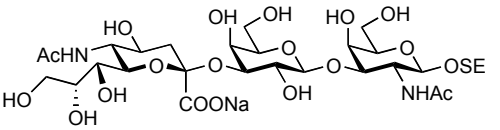
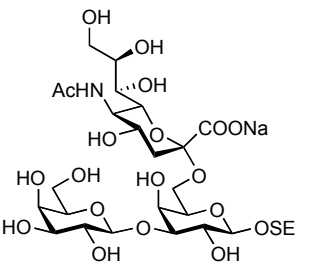
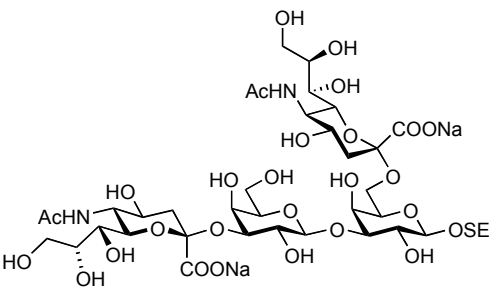
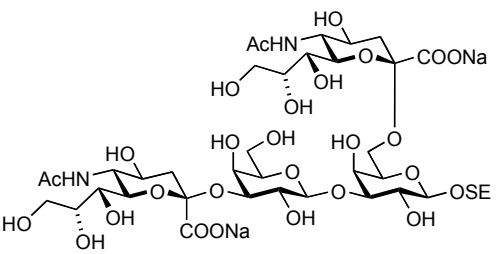
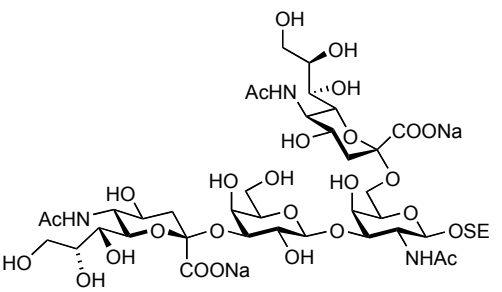
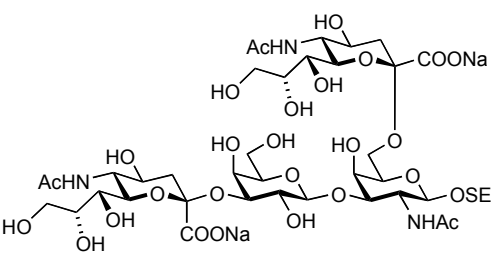
Scheme 27: a) NIS, TfOH, CH_3CN, -30°C, 16 h (45%); b) i. NaOMe, MeOH, rt, 7 h, ii. aq. NaOH, rt, 16 h (90%).

1.5. Biological assay (in collaboration with Prof. Soerg Kelm, Bremen)

By employing the fluorescent hapten inhibition assay, partial structure of GQ1bα and derivatives thereof were tested. Trisaccharide **20** served as a reference compound in this work with its rIP equal to 1, while the rIPs of the others were calculated by dividing the IC_{50} of **20** by that of tested compounds respectively. In case the test compound is more active than the reference compound, rIP > 1 is obtained. For a better comparison, besides all the oligosaccharides described above, tetrasaccharides Neu5Acα(2→3)Galβ(1→3)[Neu5Acα/β(2→6)]Gal*N*Acβ-OSE (**86α**, **86β**) were also included in *table* 8.

The inhibition studies with the synthetic compounds readily revealed several aspects of the interaction between MAG and sialylated oligosaccharides. First, α2,3-linked terminal sialic acid is the prerequisite for binding to MAG. Without it, as in trisaccharide **45**, only a weak inhibition is observed; Second, the additional α2,6-linked sialic acid residue enhanced the binding affinities, as shown by **54α** and **86α**, which were 3 to 4 times more active than **20** and **1** respectively; Third, the incorporation of a β2,6-linked sialic acid at this position leads to reduced binding as indicated by low rIPs of **54β** and **86β**; Last, there is no significant difference in the affinities when Gal*N*Ac is replaced by Gal, by comparing **20** with **1** and **54α** with **86α**. To further characterize the MAG-ligand interaction at atomic resolution, a few selected compounds were subjected to STD NMR and trNOE experiments.

Table 8: Bioaffinities of partial structures of GQ1bα and derivatives thereof.

Compound	Structure	rIPs
20		1
1		0.86
45		0.2
54α		4.2
54β		1.34
86α[a)]		3.3
86β[a)]		<0.6

a). compounds prepared by Dr. Oliver Schwardt.

1.6. STD NMR (in collaboration with Prof. Bernd Meyer)

Trisaccharides **1**, **20** and tetrasaccharide **86α** were investigated by STD NMR experiments with Fc-MAG_{d1-3} (*figure* 12). STD effects were normalized to the *N*-acetyl of the α2,3-linked terminal sialic acid, which showed the absolute effect of **1**:7.6%, **20**: 3.4% and **86α**: 24.8%, respectively. Larger values indicate closer proximity of the respective functional group to the protein and thus describe the binding epitopes.

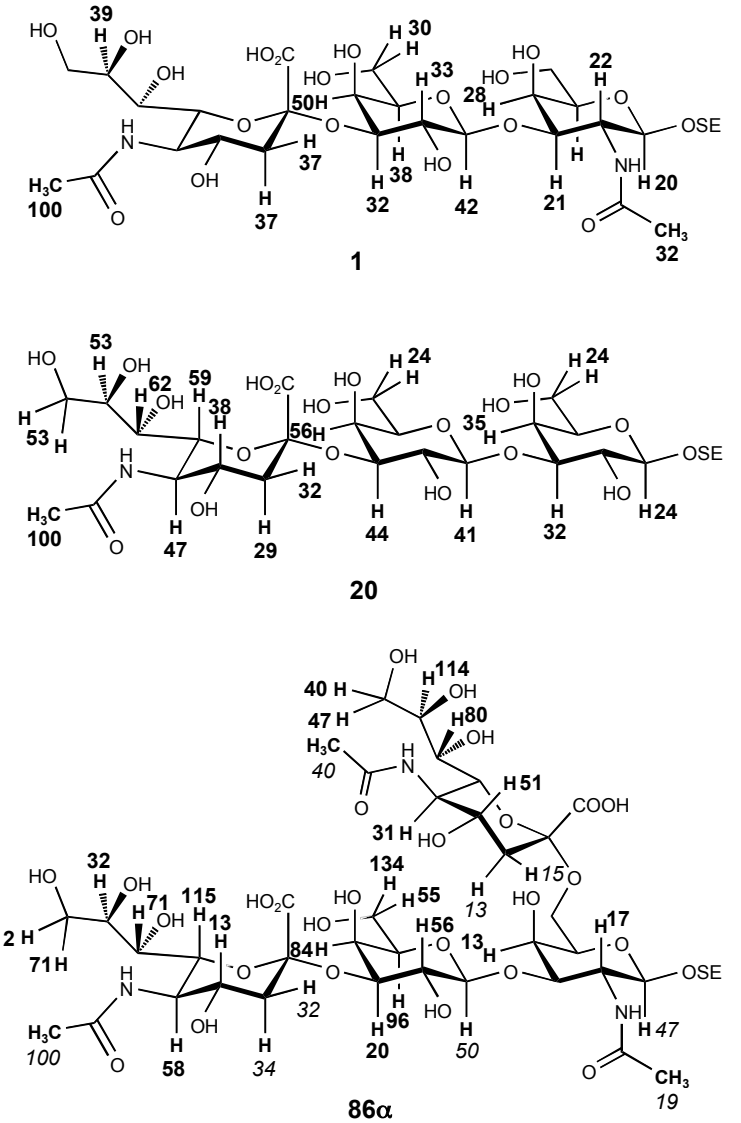


Figure 12: STD effects of **1**, **20** and **86α**.

The STD data yield information on the binding events and on the binding epitopes of the ligands upon interacting with Fc-MAG_{d1-3}. For trisaccharides **1** and **20**, it is clear that the terminal sialic acid is mostly involved in binding, while the core disaccharide

Gal-Gal*N*Ac or Gal-Gal residues should be in less intimate contact with the binding site. In the case of tetrasaccharide **86α**, both sialic acid residues are involved in binding. It should be noticed that the α2,3-linked terminal sialic acid interacts with the protein mostly *via* its *N*-acetyl group, glycerol side chain and carboxylic acid, while the last interaction is indirectly proved by the strong STD effect on 4-H of Gal, which is assumed to be caused by the salt bridge between the carboxylic acid of the sialic acid and a matching residue on the protein surface. It is especially interesting that the glycerol side chain of α2,6-linked internal sialic acid was greatly involved in binding as compared with the other functional groups within the moiety. Similar to that of the trisaccharides, the neutral Gal-Gal*N*Ac core showed low STD effects, however, the 6-H of Gal demonstrated the most pronounced effect, which indicates that the hydoxymethyl group of Gal is directly involved in the binding process. In conjunction with the results from trNOE experiments (see **1.7.**), it is likely that the trisaccharide **20** and the tetrasaccharide **86α** have a similar binding pattern.[176]

1.7. trNOE NMR (in collaboration with Prof. Thomas Peters, Lübeck)

The bioactive conformations of trisaccharide **20** and tetrasacchairde **86α** were obtained from trNOE NMR experiments. The most striking observation is the conformational change of the flexible α2,3-glycosidic linkage between terminal Neu5Ac and Gal from solution to bound conformation. In the case of trisaccharide **20**, an interglycosidic contact between Gal-H3 and Sia(I)-$H3_{ax}$ can be observed in the NOESY spectrum of the free state, which disappeared upon binding to MAG. In the presence of MAG, a contact between Gal-H3 and Sia(I)-H8 appears, suggesting a conformational change which brings these two protons near each other. The similar phenomenon was observed with sialyl $Lewis^X$ bound to E-selectin.[177] According to the trNOE data of tetrasaccharide **86α**, a similar conformational change is observed. From the two major conformations that have been described previously by several authors to be present in aqueous solution for the α2,3-glycosidic linkage the experimental trNOE data are only consistent with the so called anti-conformation. This conformation of the sialic acid linkage has also been observed in crystal structures of the Cholera Toxin complexed with the GM1 pentasaccharide, and in the

case of hemagglutinin complexed with LSTA. The analysis of the bioactive conformation of the α2,6-glycosidic linkage was not yet possible on the basis of the present data because of a heavy signal overlap, and currently is the subject to a more detailed NMR analysis.[176]

Additionally, trNOE experiments were also carried out on trisaccharide **45**. Since no build up curves were available within the mixing time, it was classified as a weaker binder to the protein, which fits with the results of the bioassay. [176]

1.8. Homology model of MAG (in collaboration with Prof. Thomas Peters, Lübeck)

In order to better understand and analyze the experimental results by NMR, it is necessary to develop a 3D model of the complexes of MAG with the ligand. The X-ray structure of sialoadhesin complexed with sialyllactose served as a starting point for the modeling and docking studies.[84] The homology model was constructed by aligning the sialoadhesin and the MAG sequences, and subsequent minimization. By docking the tetrasaccharide **86α** into the known binding pocket, a carbohydrate-MAG complexe is available as shown in *figure* 13.

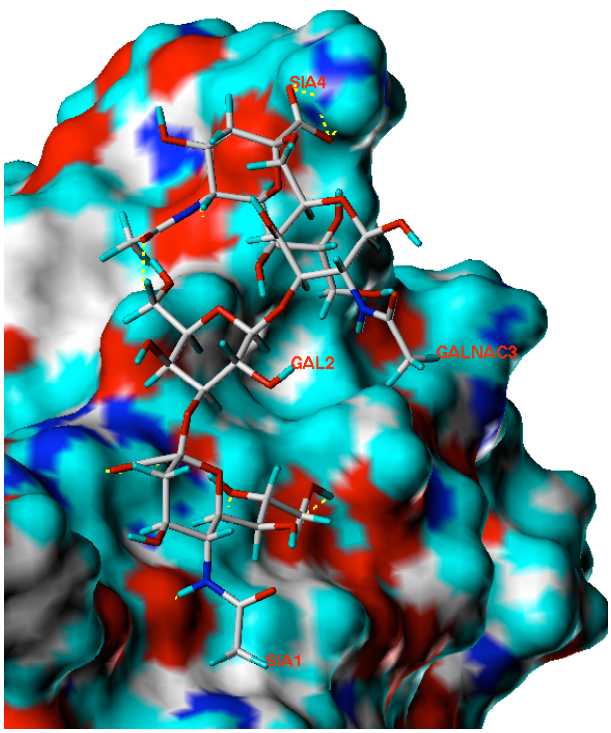


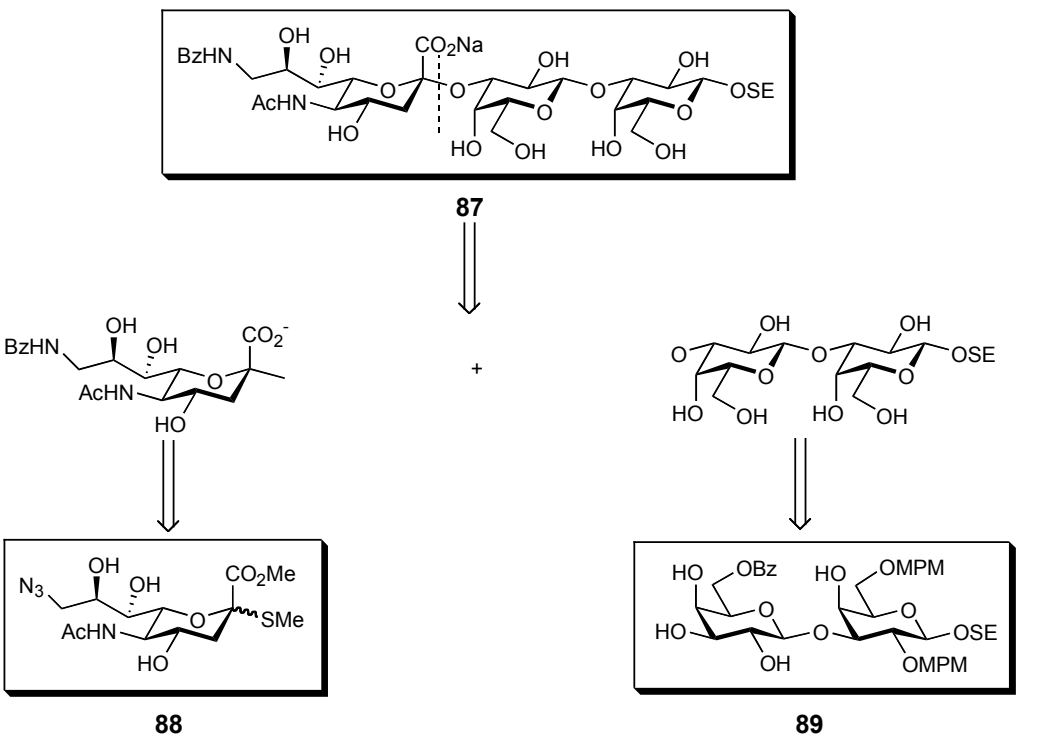
Figure 13: Homology model of MAG docking with Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)]Gal*N*Acβ-OSE (**86α**).

For the terminal α2,3-linked sialic acid residue, the most notable features include: stacking interactions of the *N*-acetyl group with Trp22, stacking interactions of its glycerol side chain with Tyr127 and an salt bridge between carboxylic acid and Arg118. In the case of internal α2,6-linked sialic acid residue, another salt bridge between carboxylic acid and Lys67, and hydrophobic and hydrogen bond interactions of the *N*-acetyl group and glycerol side chain with Tyr65 can be observed. Additionally, the hydroxymethyl group of Gal is also involved in the hydrogen bond network with Tyr65. Besides, the *N*-acetyl group of the Gal*N*Ac is oriented away from the binding pocket and should not contribute to the binding energy. It should be noted that most of the observations from this model are in good agreement with the experimental data (biological assay, STD NMR and trNOE NMR).[176]

2. Carbohydrate mimics with 9-position modified α2,3-linked terminal sialic acid

2.1. Synthesis of 9-BzNH-Siaα(2→3)-Galβ(1→3)Galβ-OSE (87)

The extremely important role of terminal α2,3-linked sialic acid for MAG recognition was in agreement with the previous studies.[56] Recognizing the dramatic increase of binding affinity achieved by the modification at 9-position of the α2,3-linked sialic acid moiety,[85] we were prompted to synthesize mimic **87**.



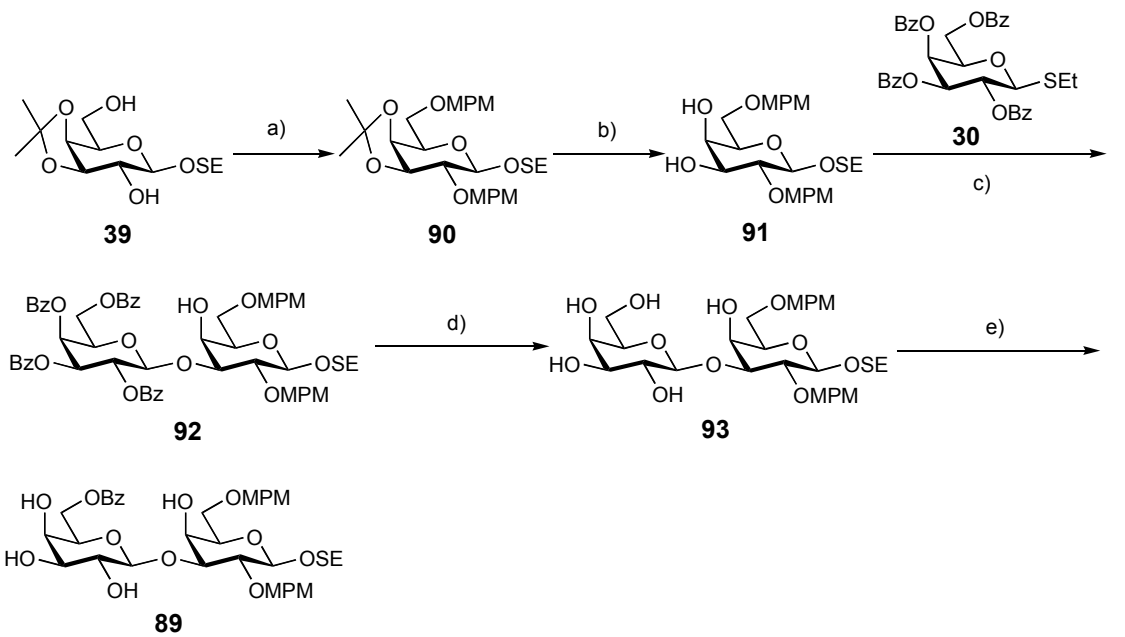
Scheme 28: Retro-synthesis of mimic **87**.

As shown in *scheme* 28, mimic **87** can be easily prepared by employing the similar strategy. The available sialyl donor **88**[178] contains an azido- group for further modifications. *para*-Methoxy phenylmethyl (MPM) was chosen to protect 2,6-position of the Gal at the reducing end of acceptor **89** due to the severe problems encountered in the debenzylations of **41** and **77**. Besides catalytic hydrogenation,[179] MPM can be cleaved selectively by various options like oxidation by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[180-182] or cerium ammonium nitrate (CAN),[183] acidic hydrolysis with camphor sulfonic acid (CSA) or trifluoroacetic acid (TFA),[184]

and electronchemical conditions,[185] *etc*., which makes it an attractive alternative to the benzyl group.

2.1.1. Preparation of disaccharide core (89)

The synthesis of disacchride **89** was started from the known building block **39** (*scheme* 29). After protecting the 2,6-OHs with MPM, using *para*-methoxy benzyl chloride (MPMCl) and NaH in DMF (→**90**, 61%), 3,4-isopropylidene group was cleaved under acidic condition to generate acceptor **91** for further glycosylation at 3-position. The subsequent coupling reaction with **30** was carried out by using DMTST as promoter in CH_2Cl_2 at 0°C, affording disaccharide product **92** with desired β-configuration in 76% yield. After removal of the benzoyl protections under Zemplén conditions (→**93**, 85%), the selective 6-*O*-benzoylation was accomplished by employing the established procedure (see **1.3.1.**), which resulted in **89** in 67% yield.

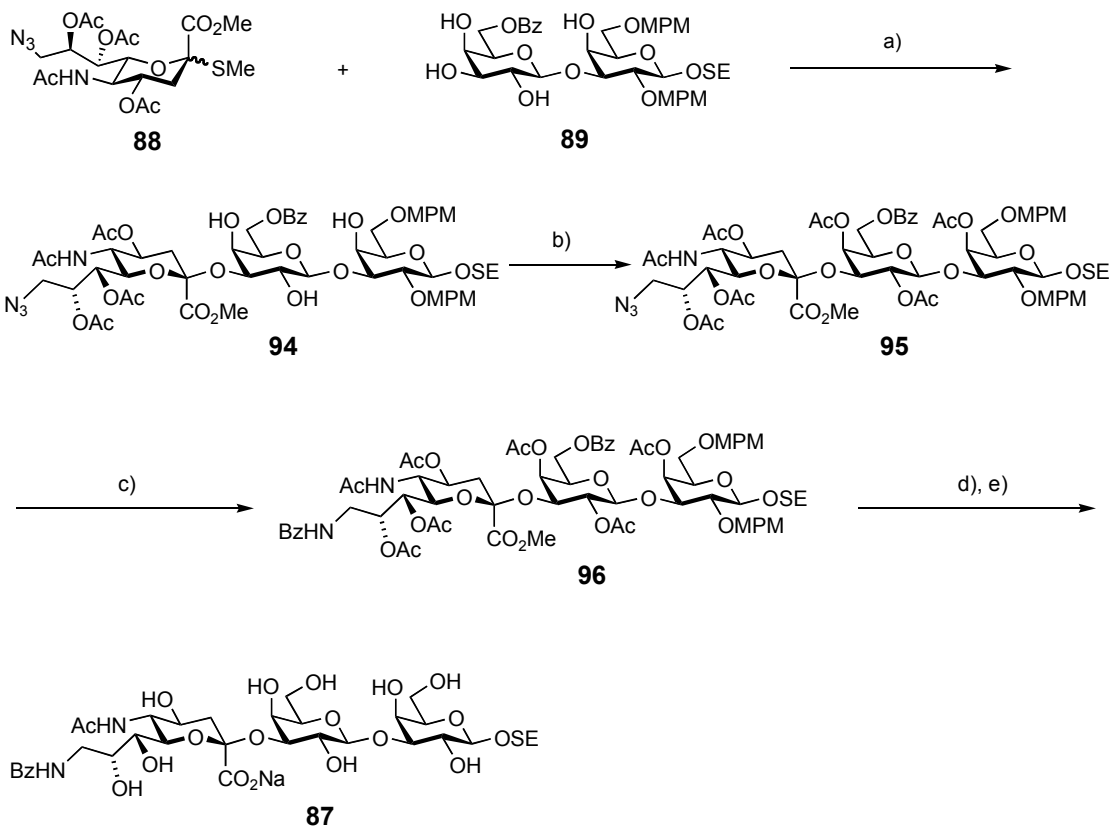


Scheme 29: a) MPMCl, NaH, DMF, 0°C, 3 h (61%); b) 80% aq. AcOH, 90°C, 1.5 h (50%); c) DMTST, CH_2Cl_2, 0°C, 16 h (76%); d) NaOMe, MeOH, rt, 2 h (85%); e) benzoyl cyanide, Et_3N-CH_3CH (1:4), -40~-45°C, 1 h (67%).

2.1.2. 9-BzNH-Siaα(2→3)-Galβ(1→3)Galβ-OSE (87)

The coupling reaction between the readily available donor **88** and **89** was carried out under the same condition as used in previous sialylations: NIS-TfOH as promoter,

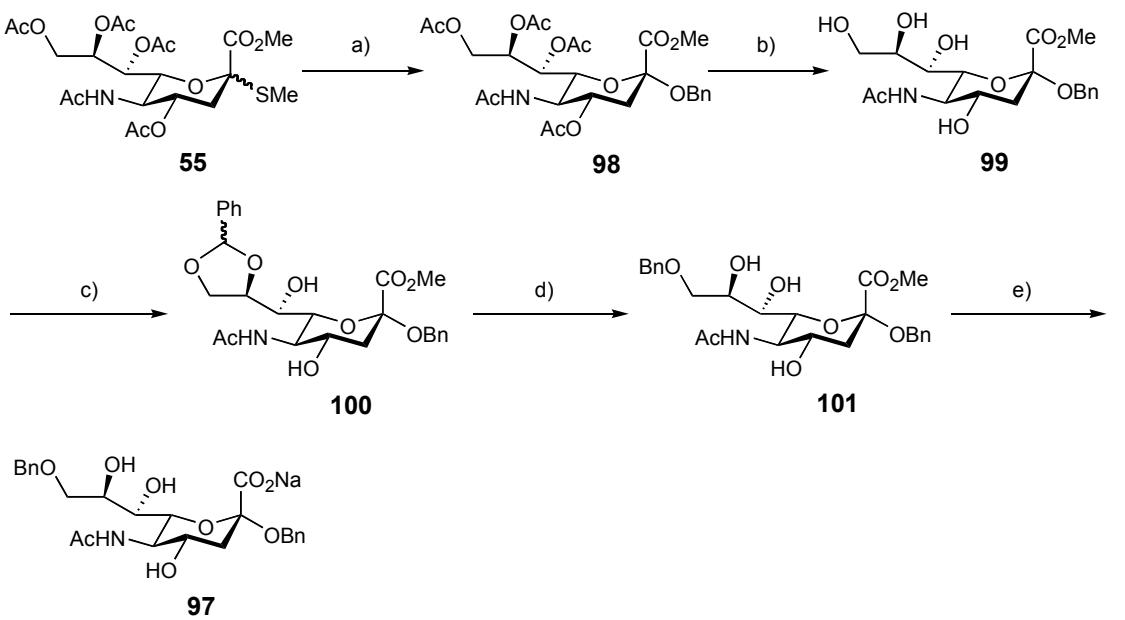
CH_3CN as solvent, at low temperature (~-30°C). Unexpectedly and repeatedly, only trace of product **94** could be obtained while the major one is the sialic acid glycal. When DMTST in CH_3CN at 0°C was tried as the promoter, an excellent yield (63%) and a good stereoselectivity (α:β=3:1) was achieved (*scheme* 30). After per-acetylation (→**95**), the azido group was converted to benzoylamide using the modified Staudinger reaction.[186] **95** was treated with triphenylphosphine (Ph_3P) in the presence of benzoyl chloride, which acylated the iminophosphorane formed in Staudinger reaction[187] to afford benzoylamide in one step (57%). MPMs were then cleaved by DDQ in CH_2Cl_2-H_2O (18:1), while the reaction with CAN in CH_3CN-H_2O (9:1) was not successful. However, the product seemed unstable in solution ($CDCl_3$). During the time of ^{13}C NMR measurement, one impurity was generated presumably due to the migration of acetyl group from 4- to 6-position in Gal moiety at the reducing end. Thus, deprotection was carried out immediately by transesterification with NaOMe in MeOH followed by hydrolysis in aq. NaOH to afford **87** in 74% yield of three steps.



Scheme 30: a) DMTST, CH_3CN, 0°C, 16 h (**94α**, 47%; **94β**, 16%); b) Ac_2O, py, DMAP, rt, 16 h (87%); c) benzoyl chloride, Ph_3P, DCE, rt, 16 h (57%); d) DDQ, CH_2Cl_2-H_2O, rt, 3 h; e) i. NaOMe, MeOH, rt, 5 h, ii. aq. NaOH, rt, 3 h (**96→87**, 74%).

2.2. Synthesis of 9-*O*-Bn-Sia (97)

After the successful synthesis of the C-9 modified trisaccharide **87**, for further investigations of the binding properities at this position, a benzyl ether functionality was proposed. It is more flexible than amide while the phenyl is retained for the possible hydrophobic interaction. Since Neu5AcαBn was found to be ten times more potent than Neu5AcαMe,[83] mimic **97** was designed to have *O*-benzyl at 9-position and another benzyl as aglycon at 2-position. As shown in *scheme* 31, sialyl donor **55** coupled with benzyl alcohol under normal condition to form **98** in 35% yield. After deacetylation with NaOMe in MeOH, regioselective protection of the 8,9-diol as a benzylidene acetal was accomplished by treatment with α,α-dimethoxytoluene in the presence of *cat*. $TsOH{\cdot}H_2O$ in CH_3CN to afford **100** as a mixture of diastereomers as indicated by ^1H NMR.[161] The benzylidene acetal of **100** was then regioselectively opened to 9-*O*-Bn with $BH_3{\cdot}NMe_3$, $AlCl_3$ in THF and trace of H_2O (**99→101**, 70%). Hydrolysis of the methyl ester took place by treating with LiOH in H_2O-dioxane solution. The subsequent conversion of the lithium salt to the sodium salt was achieved by passing through a Dowex 50×8 (Na^+) column (**97**, 87%).

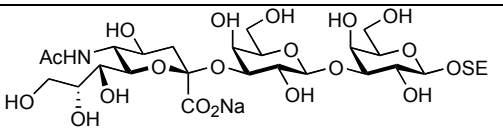
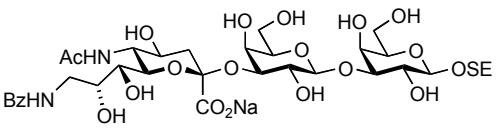
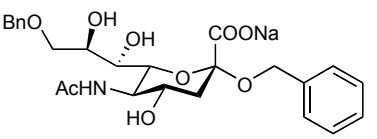
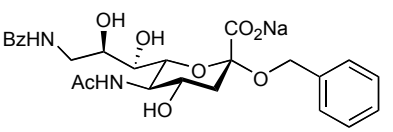
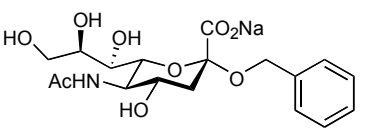


Scheme 31: a) benzyl alcohol, NIS, TfOH, CH_3CN, -35°C, 24 h (35%); b) NaOMe, MeOH, rt, 1 h (78%); c) α,α-dimethoxytoulene, $TsOH{\cdot}H_2O$, CH_3CN, rt, 1.5 h; d) $BH_3{\cdot}NMe_3$, $AlCl_3$, H_2O, THF, rt, 4 h (**99→101**, 70%); e) i. LiOH, H_2O-dioxane, rt, 1.5 h, ii. Dowex 50×8 (Na^+) (87%).

2.3. Biological assay

With the fluorescent hapten inhibition assay, mimics **87** and **97** were tested together with reference **20**. The resulting rIPs are listed in *table* 9, which also included compounds **102** and **103** for comparison.

Table 9: Bioaffinities of mimics with C-9 modified α2,3-linked sialic acid.

Compound	Structure	rIPs
20		1
87		203
97		2.3
102[a)]		690
103[a)]		0.62

a). compounds prepared by Sachin Shelke, Institute of Molecular Pharmacy, University of Basel.

According to the rIPs, trisaccharide mimic **87** with the only modification at 9-position of the sialic acid residue achieved 203 times of affinity over the reference **20**. However, it is about 3 times weaker than its monosaccharide analogue **102**, which has a benzyl group as aglycon instead of disaccharide core. It is surprising since the previous studies[79] demonstrated that the additional contacts of the neutral core with the protein contribute to the binding potency as well, although to a lesser degree, which is also confirmed in this work either by biological data (e.g. **1** and **103**), or by

STD NMR effects observed on the core structure. Possibly, the dramatically improved affinity of **102** might be caused by "induced fit" of the protein upon binding. The same reason may also explain the relative low binding affinity of compound **87**, in which the disaccharide core is hindered from binding by the change of the protein conformation.

Although monosaccharide **97** has a 2.3 times higher affinity than **103**, the effect of the substitution by a benzyl ether is not comparable to the dramatic enhancement by benzoylamide replacement of 9-OH **102**. It is likely that the hydrogen bond interaction is very crucial for the binding since one difference between benzoylamide and benzyl ether is that the former can serve as a hydrogen bond donor and acceptor while the latter acts only as an acceptor.

3. Mimics with modifications of the disaccharide core

In contrast to the important contribution of the two sialic acids to binding, numerous SAR studies[56,79] indicate that the disaccharide core behaves predominantly as a linker holding the two sialic acid moieties in the desired spatial orientation. Therefore, the core is a good point to develop mimics.

It has long been recognized that aromatic moieties are major players in molecular recognition.[188] For example, the protein binding of drugs that contain aromatic substituents is dominated by interactions with aromatic and hydrophobic residues.[189] As a consequence, aromatic and hetero aromatic moieties such as benzene, pyridine, pyrimidine, were found to be the most commonly used elements in a retrospective statistical analysis of privileged scaffolds of drugs.[190] In addition, aromatics and hetero aromatics have been shown to form favorable interactions with polar substituents, such as amides[191] or hydroxyl groups,[192] and even positively charged moieties.[193,194] Therefore, they can serve as good candidates for carbohydrate scaffolds. Besides the simplified structure and possibly increased binding affinity, benefits caused by the substitution of carbohydrates by aromatics also include easier

synthetic access, increased thermal and chemical stability, as well as the enhanced bioavailability, all of which are essential for pharmacologically active molecules to become drugs.

3.1. Phenoxyphenyl(methyl) and biphenyl(methyl) derivatives of sialic acid

It was reported by Kelm *et al.*[83] that Neu5AcαBn has a ten-fold higher affinity for MAG than the corresponding methyl sialoside, suggesting an additional hydrophobic interaction of the benzyl residue with the receptor site. To further investigate such interaction, and to test the spacer function of the disaccharide core, phenoxyphenyl and biphenyl scaffolds were chosen for the substitution of the Galβ(1→3)Gal moiety. Both phenoxyphenyl and biphenyl are privileged moieties that can be commonly found in drugs.[190] It is interesting to note that biphenyl substructures are found in 4.3% of all known drugs, and various therapeutic areas (*e.g.* diflunisal and valsartan).[188] A statistical analysis of NMR-derived binding data on 11 protein targets indicates that the biphenyl motif, over naphthyl, diphenylmethane, or bibenzyl, is a preferred substructure for protein binding.[188] This preference for biphenyls was rationalized by the degree of flexibility around the aromatic linkage. The naphthyl group is completely rigid, which severely limits the number and type of pockets on the protein site it can occupy, while the greater flexibility of the bibenzyl and diphenylmethane substructures may result in entropic penalties upon binding.[188]

Therefore, mimetics **104**-**107** (*figure* 14) were designed with phenoxyphenyl(methyl) and biphenyl(methyl) as aglycons. **107** has an extra acetic acid attached on the biphenyl moiety to mimic the α2,6-linked internal sialic acid, the carboxylic acid of which was supposed to contribute to MAG-binding substantially.[75]

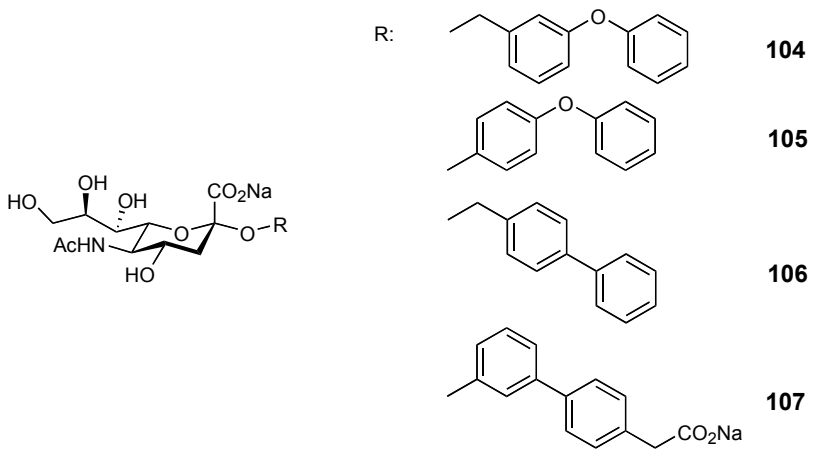
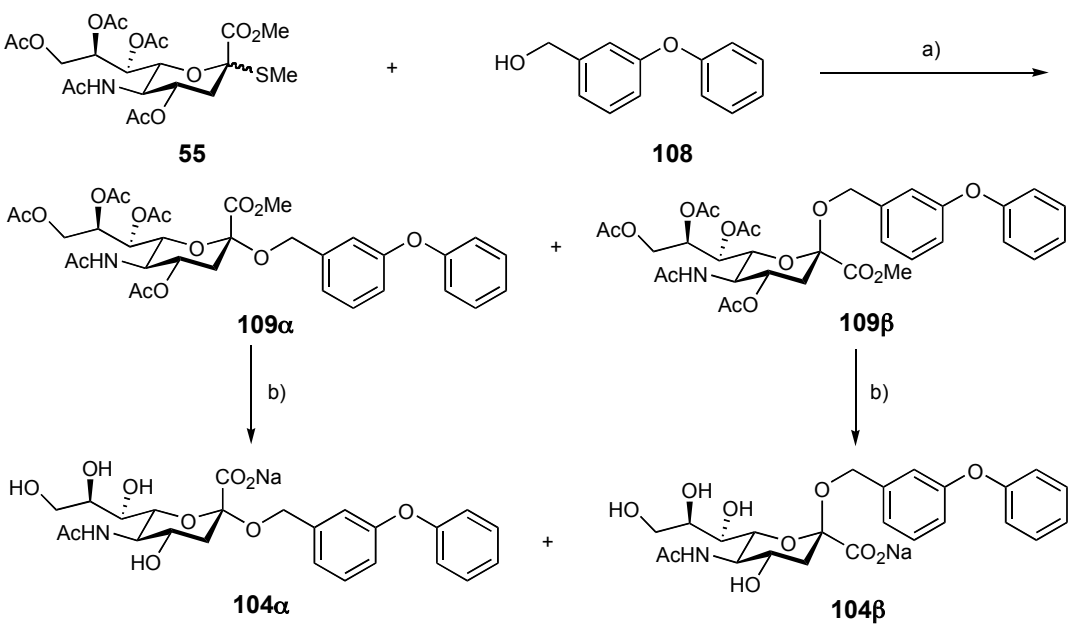
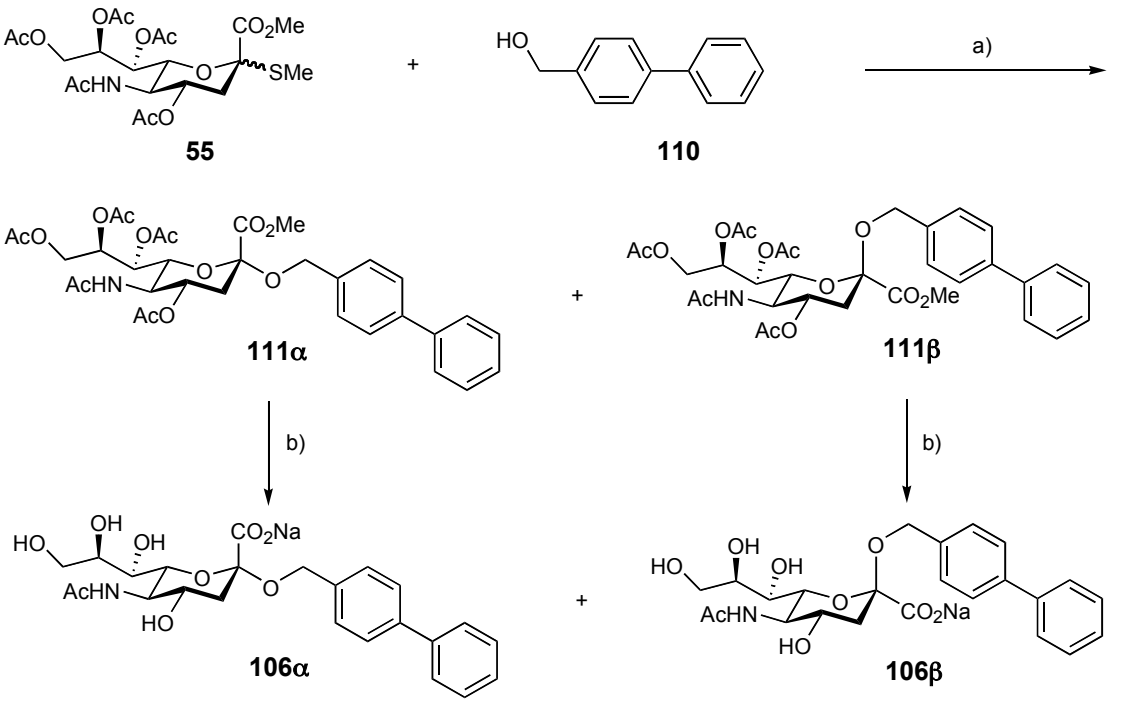


Figure 14: Phenoxyphenyl(methyl) and biphenyl(methyl) derivatives of sialic acid.

As shown in *scheme* 32, the preparation of **104** was very straightforward by coupling the common sialyl donor **55** with 3-phenoxybenzyl alcohol (**108**) by the activation with NIS-TfOH in CH_3CN at –30°C. Product **109** was obtained of 81% yield with a ratio of 2.5:1 (α:β). Transesterification with NaOMe in MeOH followed by hydrolysis in aq. NaOH afforded **104α** and **104β** in 60% and 63% yields, respectively. Similarly, the coupling reaction of **55** with 4-biphenyl methanol (**110**) was carried out with the exception that TfOH was replaced by TMSOTf. **111** was obtained with the anomeric stereoselectivity 3:1 (α:β) in 89% yield. The final deprotection was accomplished in two steps in about 60% yield for both anomers (**106α** and **106β**, *scheme* 33).

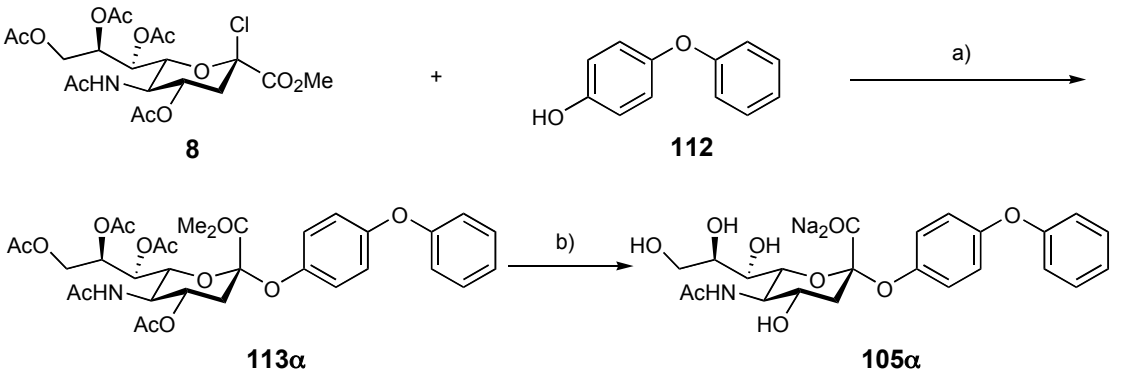


Scheme 32: a) NIS, TfOH, CH_3CN, -30°C, 16 h (81% **109α**:**109β**=2.5:1); b) i. NaOMe, MeOH, rt, 4 h, ii. aq. NaOH, rt, 16 h (**104α**, 60%; **104β**, 63%).



Scheme 33: a) NIS, TMSOTf, CH_3CN, -30°C, 16 h (89% **111α**:**111β**=3:1); b) i. NaOMe, MeOH, rt, 4 h, ii. aq. NaOH, rt, 16 h (**106α**, 60%; **106β**, 57%).

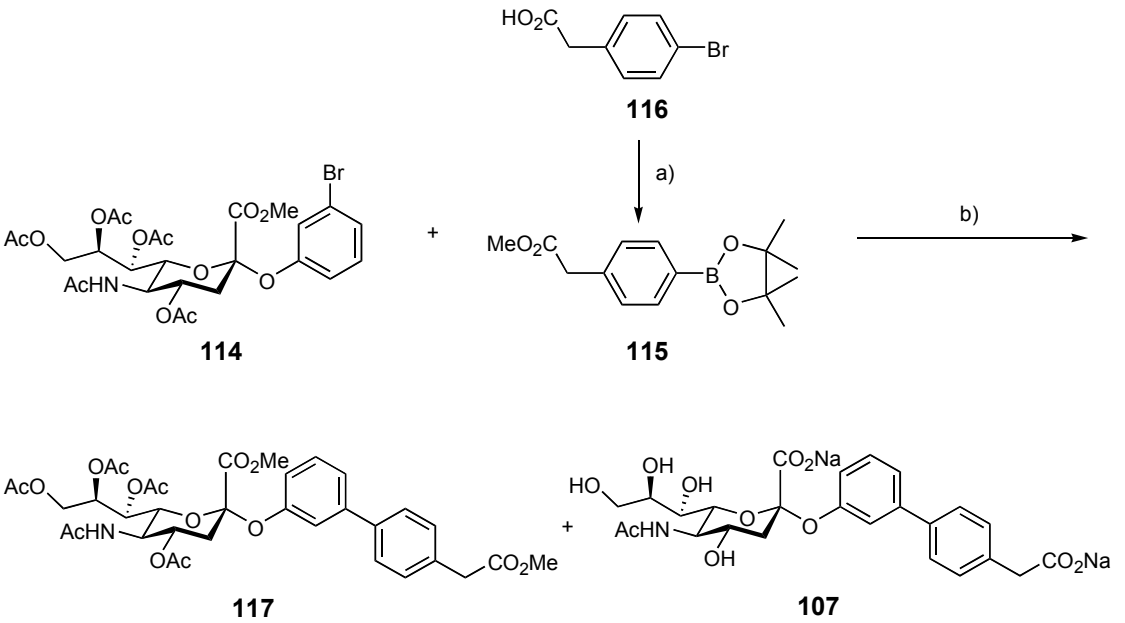
It should be noted, that in contrast to the sialylations of the benzyl alcohols, the coupling reaction between sialyl donor **55** and 4-phenoxyphenol (**112**) under identical conditions failed. With the NIS-TfOH promoter system, no product was formed according to TLC. Meanwhile, when DMTST was employed as promoter in CH_3CN at 0°C, trace of product (10%) with a reversed stereoselectivity (α:β=1:2) as determined by ^{1}H NMR (data was not shown) were obtained. The failure was mostly due to the low nucleophilicity of phenol by comparing with benzyl or sugar alcohols, which normally react well under the traditional coupling conditions. Surprisingly, there are only few methods described in the literature for the formation of aryl sialosides. One of the most frequently used procedures employs phase transfer conditions starting from phenols with 2-chloro derivatives of sialic acid.[195] As shown in *scheme* 34, the 2-chloro sialyl donor **8** was refluxed with 4-phenoxyphenol (**112**) in chloroform-aq. NaOH in the presence of benzyltriethylammonium chloride, affording desired α-anomer **113α** in 60% yield. Subsequent deprotection gave **105α** in 81% yield.



Scheme 34: a) $BnEt_3N^+Cl^-$, NaOH, CH_3Cl-H_2O, reflux, 2 h (60%); b) i. NaOMe, MeOH, rt, 5 h, ii. aq. NaOH, rt, 16 h (81%).

The Suzuki reaction was investigated for the synthesis of mimetic **107** (*scheme* 35), which can then serve as a general method to prepare various substituted biphenyls for SAR study. Sialic acid derivative **114** was prepared by phase transfer reaction of 2-chloro sialyl donor **8** with *meta*-bromo-phenol[196]. The arylboronic ester **115** was achieved in two steps. Esterification of **116** in the presence of CAN in MeOH was followed by palladium-catalysted cross coupling reaction namely Miyaura boronation,[197] which was carried out in the presence of $PdCl_2$(dppf), dppf and KOAc in dioxane. It should be noted that the reaction was greatly speeded up under microwave (300W) radiation, which afforded the boronic ester **115** in 50% yield.

In a systematic exploration of the Suzuki reaction with the similar substances,[196] factors as base, catalyst, ligand and reaction conditions (*e.g.* solvent, temperature, microwave) were optimized. This optimal procedure was employed in *scheme* 35. Since the reaction was extremely slow under normal condition, side reaction happened during extensive heating. Microwave radiation substantially accelerated the reaction rate at high temperature. Even though, only 8% of the desired product **117**, along with the deprotected substance **107** (3%) and recovered starting material **114** (25%)were obtained after cumbersome chromatographic separation.



Scheme 35: a) i. CAN, MeOH, rt, 16 h (90%), ii. bis(pinacolato)diboron, $PdCl_2$(dppf), dppf, KOAc, dioxane, 120°C (mocrowave 300W), 1.5 h (50%); b) $PdCl_2$(dppf), dppf, BHT, K_3PO_4, dioxane, 170°C (microwave 300W), 2.25 h (**117**, 8%; **107**, 3%).

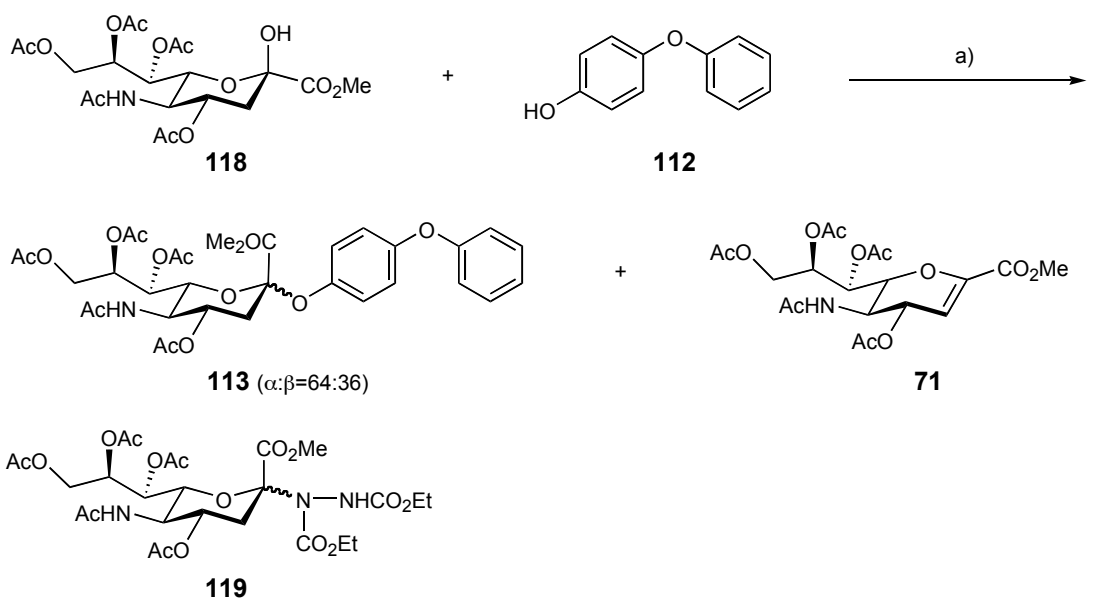
3.2. Development of Mitsunobu procedure for the synthesis of aryl sialosides

In contrast to aldoglycosides, only few methods for the stereoselective synthesis of ketoglycosides, such as aryl sialosides, exist.[195] Besides the phase transfer reaction employed for the synthesis of **105α**, Williamson ether synthesis based on phenolates and 2-halogeno derivatives of sialic acid is another choice, although the low solubility of sodium phenolates drastically limits the scope of this method.[198,199] Both approaches result in inversion of the anomeric configuration of the sialyl donor and give moderate to good yields. However, side reactions caused by the basic reaction conditions, e.g. hydrolysis of protecting groups or glycal formation by 2,3-elimination, are inevitable.

Meanwhile, the Mitsunobu reaction[200] is widely used for the synthesis of aryl,[201-210] acyl,[209-212] alkyl,[209,213,214] and amino glycosides[209,215] by reacting hemiacetals of aldosugars, predominantly glucose, galactose and mannose, with weakly acidic acceptors in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (Ph_3P). A major advantage of this direct dehydrative coupling procedure is that activated glycosyl donors do not need to be isolated and all reaction steps – anomerization, activation and glycosidic bond formation – occur in a single series of

events.[216] The stereochemical outcome depends on the anomeric ratio present in the hemiacetal and its mutarotation occurring under reaction conditions. Phenolic nucleophiles are good acceptors for this glycosylation reaction with aldosugars, leading to aryl glycosides.[201,206,208]

To our knowledge, Mitsunobu conditions have not been applied to glycosylations with ketosugars such as sialic acid, probably because their anomeric centers were regarded as being too sterically hindered.[217] However, a limited number of successful non-carbohydrate cases with comparable steric circumstance have been reported, albeit with moderate yields and stereoselectivities.[218-220] Therefore, Mitsunobu conditions were applied to the glycosylation reaction with sialyl hemiacetal **118** and 4-phenoxyphenol (**112**). Gratifyingly, after the exploration of various reaction conditions, such as solvent, temperature, order of addition and additives, the optimized procedure as shown in *scheme* 36 was developed. Hemiacetal **118**[221] predominantly adopting β-configuration[222,223] was reacted with 4-phenoxyphenol (**112**) in the presence of Ph_3P, DEAD in CH_3CN at 0°C, affording product **113** in 75% isolated yield (α:β=64:36) with traces of byproducts **71** and **119**, which have been formed from 2,3-elimination and addition of DEAD, respectively.



Scheme 36: a) Ph_3P, DEAD, CH_3CN, 0°C, 3 h (**113**, 75%; **113**:**71**:**119**=1:0.3:0.1).

To avoid a cumbersome chromatographic separation from the sialic acid glycal,[132,159] which is formed as a side-product, an oxidative work-up procedure was employed. By treating the crude product with 2-3 equiv. of $NaIO_4$ in the presence of a catalytic amount of $RuCl_3$ for only 2-5 min, the glycal was selectively oxidized and the resulting polar products were then readily removed by extraction with water.

To evaluate the optimized reaction conditions and oxidative work-up procedure, 10 other phenols were tested. In most cases, high yields and α/β-selectivites in the range of 2 : 1 were obtained. It is noteworthy that the yields correlate with the acidity of the glycosyl acceptors. Phenols bearing electron-withdrawing substituents gave excellent yields, while phenols substituted with electron-donating groups resulted in lower yields and increased amounts of byproducts.[224]

3.3. Phenyltriazole derivatives of sialic acid

Recently, the copper-(I)-catalyzed 1,2,3-triazole formation from azides and acetylenes which is a modification of Huisgen 1,3-dipolar cycloaddition attracted increasing attention.[225] Besides all the advantages of click chemistry[226] it possesses, its triazole products are interesting molecules for drug discovery. They are more than just passive linkers; they are able to interact with biological targets through hydrogen bonding and dipole interactions. In addition, triazoles do not undergo hydrolytic cleavage reactions, and are nearly impossible to oxidize or reduce, which is a desired properity of a pharmacophore.[227]

In our study, the extremely low yield of the Suzuki coupling reaction in the synthesis of biphenyl derivative **117** made the development of diverse mimetics of this family quite cubersome. The idea of employing phenyltriazole moiety as the spacer instead of biphenyl was greatly supported by *in silico* considerations (*figure* 15). The superimposition of the two molecules showed a remarkable identity, since the acid moieties mimicking the α2,6-linked sialic acid have the same spatial orientation. Therefore, three phenyltriazole sialic acid derivatives **120**-**122** were synthesized (*figure* 16).

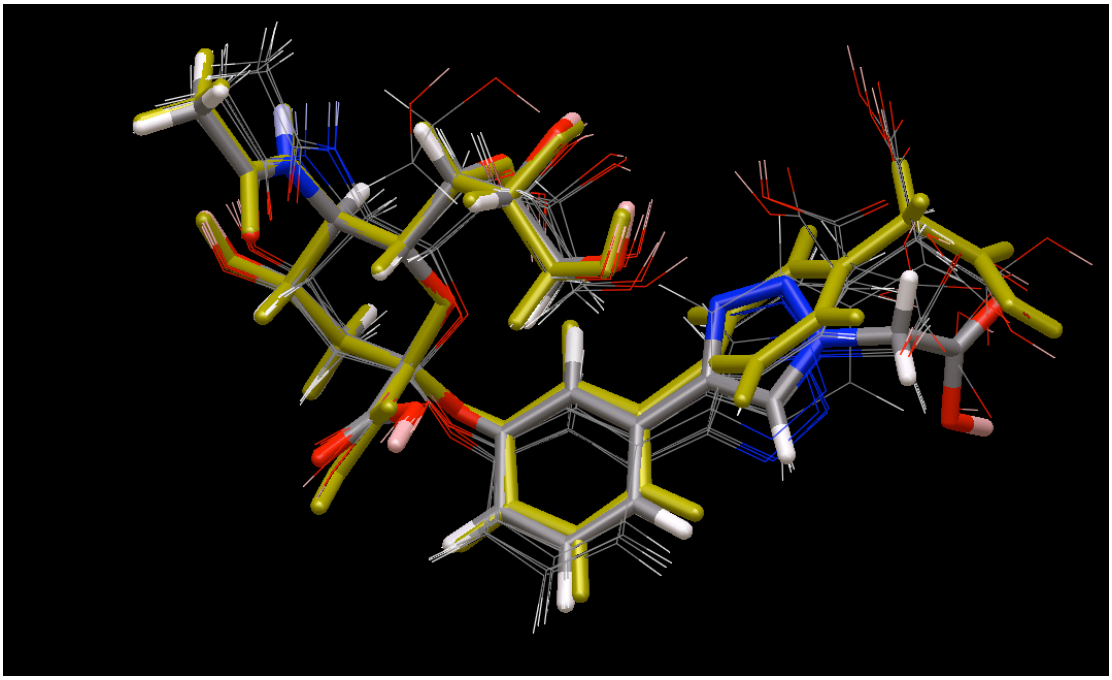


Figure 15: Superimposition of biphenyl and phenyltriazole sialic acid derivatives (**107** and **121**). Mimic **107** is in brown, while mimic **121** is shown in colors according to the atom type.

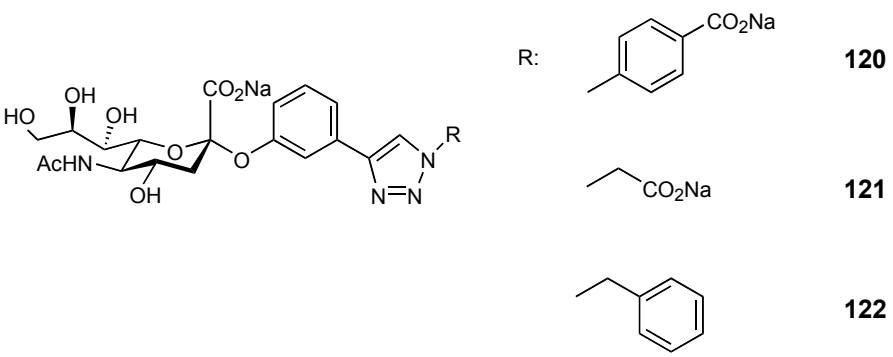
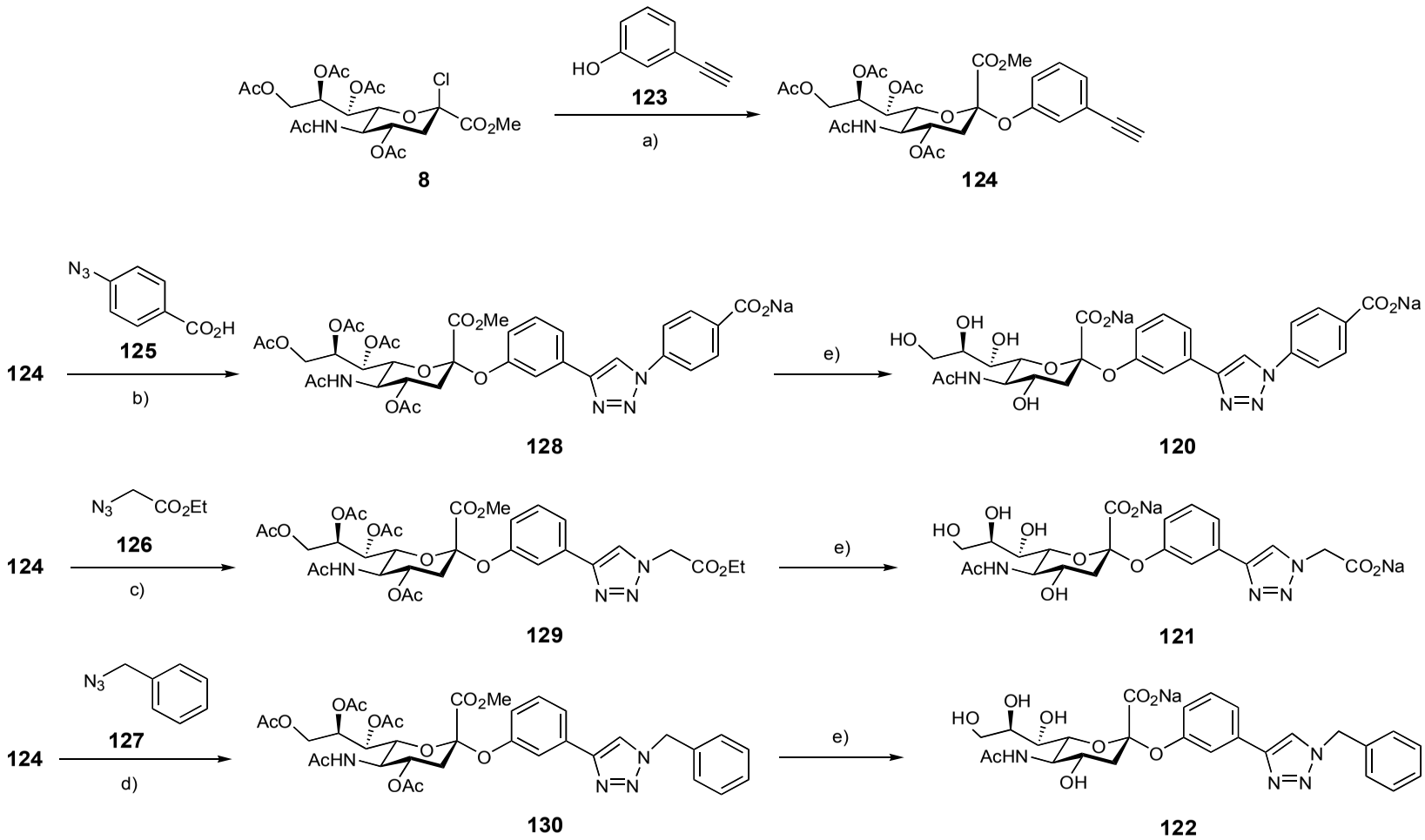
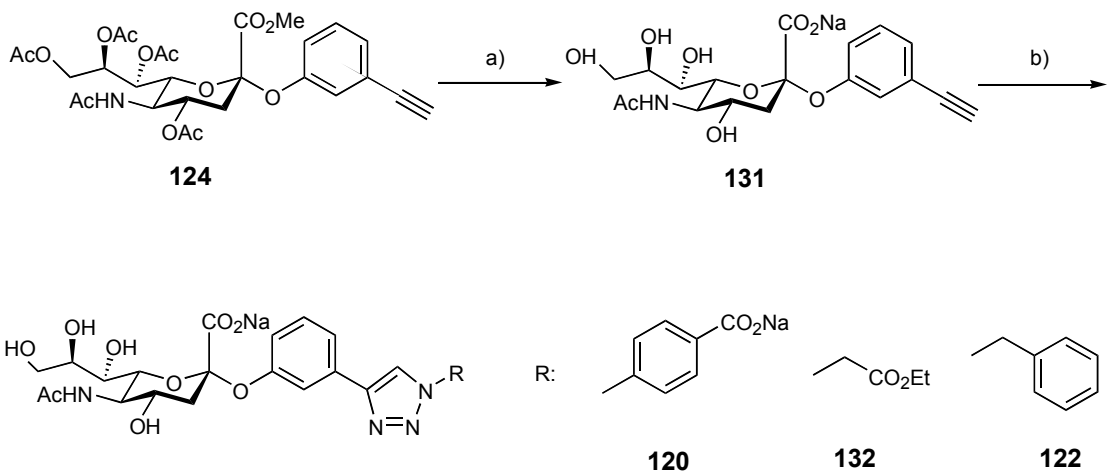


Figure 16: phenyltriazole sialic acid derivatives **120**-**122**.



Scheme 37. a) $BnEt_3N^+Cl^-$, NaOH, CH_3Cl-H_2O, reflux, 2 h (60%); b) sodium ascorbate (0.2 equiv.), $CuSO_4 \cdot 5H_2O$ (0.02equiv.), ^{t}BuOH-H_2O, 20 h (75%); c) sodium ascorbate (0.4 equiv.), $CuSO_4 \cdot 5H_2O$ (0.04 equiv.), ^{t}BuOH-H_2O, 48 h (41%); d) sodium ascorbate (1.0 equiv.), $CuSO_4 \cdot 5H_2O$ (0.1 equiv.), ^{t}BuOH-H_2O, 24 h (68%); e) NaOMe, MeOH, rt, 4h, ii. aq. NaOH, rt, 16 h (**120**, 81%; **121**, 97%; **122**, 87%).

As shown in *scheme* 37, phenylacetylene derivative **124** was obtained by a phase transfer reaction of 2-chloro sialyl donor **8** and 3-hydroxyphenylacetylene (**123**). Refluxing in $CHCl_3$-aq. NaOH in the presence of benzyltriethylammonium chloride afforded desired α-anomer **124** in 60% yield. Subsequent copper-(I)-catalyzed 1,2,3-triazole formation from azides **125**, **126** and **127**, and acetylene **124** were carried out under standard conditions.[225] Surprisingly, in all the reactions, the starting material **124** was never consumed completely under the standard condition. Sodium ascorbate and $CuSO_4 \cdot 5H_2O$, which generate Cu(I) *in situ* had to be repeatedly added till the completion of the reaction. The possible reason might be, instead of being reduced to Cu (I) and then taking part into the catalysis cycle, Cu^{2+} form the complex with the acetyls, which hinder the reaction. To prove this, acetylene **124** was fully deprotected, then coupled with the azides **125**-**127** (*scheme* 38). With catalytic amount of sodium ascorbate (0.1 equiv.) and $CuSO_4 \cdot 5H_2O$ (0.01 equiv.), phenyltriazole sialyl derivatives **120**, **132**, **122** were achieved in around 90% yields.



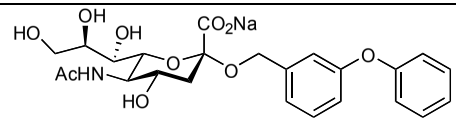
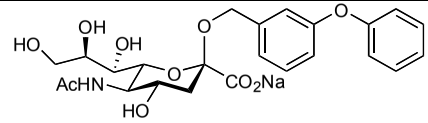
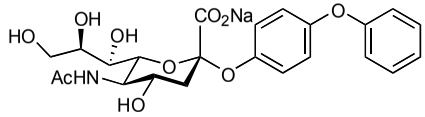
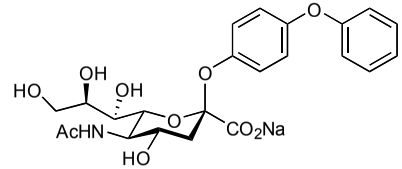
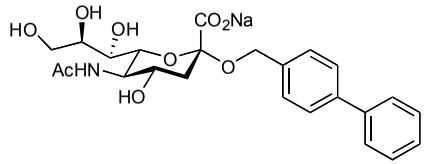
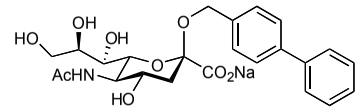
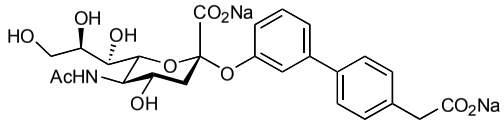
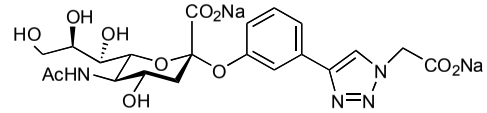
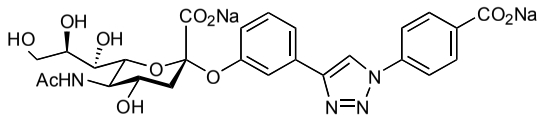
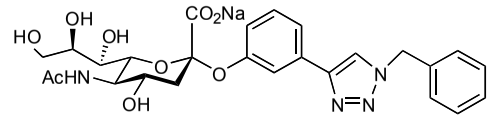
Scheme 38: a) i. NaOMe, MeOH, rt, 2.5 h, ii. aq. NaOH, rt, 2.5 h (84%); b) **125**-**127**, sodium ascorbate (0.1 equiv.), $CuSO_4 \cdot 5H_2O$ (0.01equiv.), ^tBuOH-H_2O, 16 h (**120**, 87%; **132**, 93%; **122**, 89%).

3.4. Biological assay

With the trisaccharide **20** as reference, the two families of mimics were tested by fluorescence hapten inhibition assay as shown in *table* 10. Firstly, comparable bioaffinities were obtained with the modification of natural disaccharide core by phenoxyphenyl(methyl) and biphenyl(methyl) moieties (**104α**-**106α**), while the β-anomers showed no binding affinities. Especially, mimic **105α** achieved rIP of 0.89,

suggesting the retained affinity by comparing with the reference. Secondly, phenyltriazole behaves as a good substitute of the biphenyl linkage, which is proved by the similar rIPs of **107** and **121** (1.0 and 1.1). Lastly, the unexpected high affinity of mimic **122** (rIP 1.7) implies that instead of the anionic charge, which was supposed to be the key to enhance the binding affinity of α2,6-linked sialic acid moiety,[75] a hydrophobic interaction might be the main contributor to the biniding with MAG.

Table 10: Bioaffinities of the mimics with modifications of the disaccharide core.

Compound	Structure	rIPs	Compound	Structure	rIPs
104α		0.27	**104β**		n.a.
105α		0.89	**105β**		n.a.
106α		0.38	**106β**		n.a.
107		1.0	**121**		1.1
120		0.9	**122**		1.7

Conclusion and Outlook

1. Summary of the thesis

Gangliosides, such as GD1a, GT1b and GQ1bα, were identified as specific functional ligands responsible for MAG-mediated inhibition of neurite outgrowth. MAG, the only siglec of the mammalian CNS binds gangliosides with high specificity. Based on the previous SAR studies, partial structure of natural ligands, derivatives and mimics thereof were chemically and chemo-enzymatically synthesized in this thesis. The resulting information from biological assay and NMR based conformational analysis gives guidance for the rational design of novel MAG antagonists.

1.1. Synthesis of the partial structure of natural ligands and derivatives thereof

Trisaccharides **1**, **20**, **45** and tetrasaccharides **54α**, **54β** were chemically or chemo-enzymatically prepared to investigate the respective contribution of α2,3-linked and α2,6-linked sialic acid to the affinity for MAG (*figure* 17). Compounds **1**, **45**, **54α**, **54β** were chemically synthesized using a 2-methylthio sialyl donor, and NIS-TfOH as a promoter. Trisaccharide **20** was synthesized chemo-enzymatically on a preparative scale by using *rST3Gal III*, which transfers the sialic acid moiety from CMP-Neu5Ac to the non-natural substrate Galβ(1→3)Galβ-OSE.

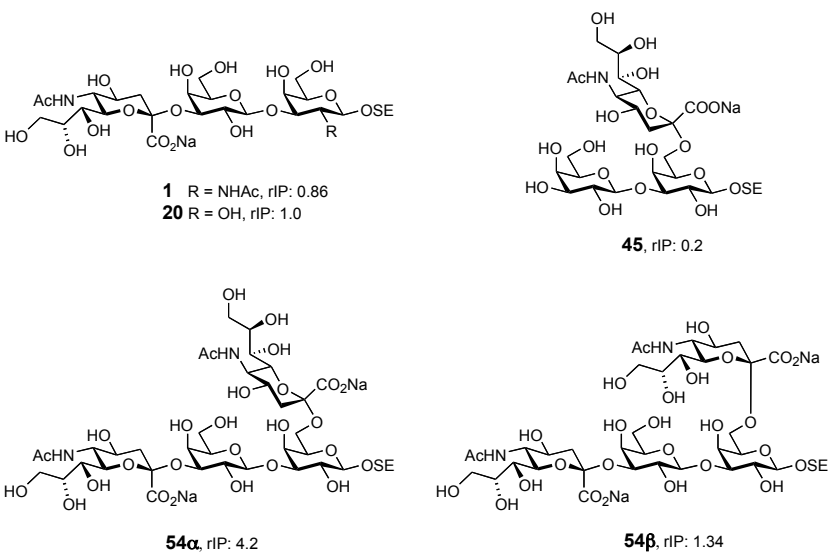
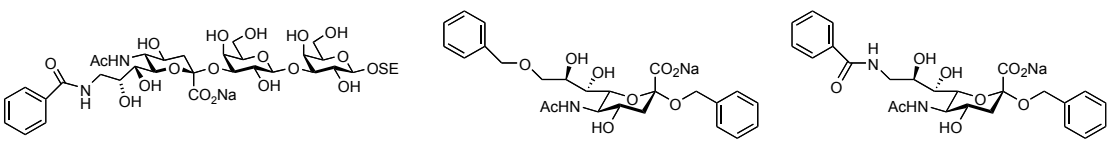


Figure 17: Partial structure of natural ligands and derivatives thereof.

Results from the biological assay and NMR studies show that the α2,3-linked terminal sialic acid is the primary request for MAG-binding, which interacts *via* its *N*-acetyl group, the glycerol side chain and the carboxylic acid with the protein. The additional α2,6-linked internal sialic acid, but not its β-anomer, can further enhance the binding affinity. Retained or even better affinities were achieved by replacing the disaccharide core Galβ(1→3)Gal*N*Ac by Galβ(1→3)Gal, which suggests that it acts predominantly as a spacer.

1.2. Synthesis of carbohydrate mimics with C-9 modified α2,3-linked sialic acid

Recognizing the important role of the terminal α2,3-linked sialic acid for MAG-binding and the dramatic improvement of binding affinity achieved by C-9 modifications of this moiety, mimics **87** and **97** were synthesized (*figure* 18).



87, rIP: 203 **97**, rIP: 2.3 **102**, rIP: 690

Figure 18: Carbohydrate mimics with C-9 modified sialic acid.

According to the biological evaluation, mimic **87** is 203 times more potent than the reference compound **20**, but about 3 times weaker than its monosaccharide analogue **102**. Surprisingly, mimic **97** is only 2.3 times better than **20**, suggesting that the benzoylamide functionality at C-9 extremely increases the potency of the ligand for MAG-binding. The dramatically improved affinity of **102** might be caused by "induced fit" of the protein upon binding. The same reason may also explain the relative low binding affinity of compound **87**, in which the disaccharide core is hindered from binding by the change of the protein conformation.

1.3. Synthesis of carbohydrate mimics with modifications of disaccharide core

To elucidate the hypothesis of the "spacer" role of the disaccharide core, it was replaced by phenoxyphenyl(methyl) and biphenyl(methyl) (*figure* 19). Mimics **104**

and **106** were synthesized using the standard sialylation procedure, while the aryl sialoside **105** was prepared in an excellent yield and with good stereoselectivity by Mitsunobu reaction, which to the best of our knowledge, was used for the first time for the glycosylation of ketosugars. The Suzuki reaction was employed for the synthesis of the substituted biphenyl derivative **107**. Results from the bioassay confirmed the "spacer" role of the disaccharide, showing the similar affinities of the mimics to that of the reference compound **20**. It should be noted that these mimics are structurally extremely simplified. In addition, the aromatic linker is expected to improve their pharmacokinetic properties compared to natural carbohydrate ligands.

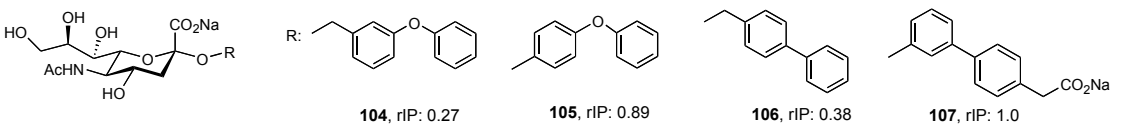


Figure 19: Phenoxyphenyl(methyl) and biphenyl(methyl) derivatives of sialic acid.

Due to the low yields of the Suzuki reaction, which made the development of various substituted biphenyl sialyl derivatives for further SAR study quite cumbersome, another linkage, namely phenyltriazole, was investigated based on its similar conformation *in silico*. Mimics **120**-**122** (*figure* 20) were obtained by a modified Huisgen 1,3-dipolar cycloaddition with high yields. Their binding affinities showed that phenyltriazole is a good substitute of the biphenyl linkage.

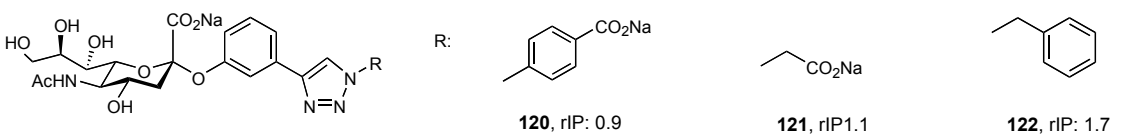


Figure 20: Phenyltriazole derivatives of sialic acid.

2. Outlook

Compared with the rIP of **120**, the unexpected high affinity of mimic **122** (rIP: 1.7) implies that instead of the anionic charge, which was supposed to be the key to

enhance the binding affinity of α2,6-linked sialic acid moiety, a hydrophobic interaction might be the main contributor of Sia (II) to the binding with MAG. Similar results were obtained in another study where Gal at the reducing end of **20** was substituted by 1,2-dideoxy-Gal.[228] By using the same reference **20**, the rIP of mimic **133** with a benzyl ether instead of Sia (II) is 2.0, while mimic **134** containing a biphenyl substitutuion achieves its rIP of 4.1, which is comparable with those of tetrasaccharides **54α** and **86α** (4.2 and 3.3 respectively) (*figure* 21*)*. Additionally, the homology model of MAG with Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)] Gal*N*Acβ-OSE (**86α**) indicates a salt bridge between Lys67 and carboxylic acid of the α2,6-linked internal sialic acid. To further elucidate the substructural specificity of Sia (II) to MAG binding, Fc-MAG_{d1-3} with the mutation of Lys67 to Ala (Fc-MAG_{d1-3} K67A) was employed in the biological assay, which showed no significant difference from the wild type.

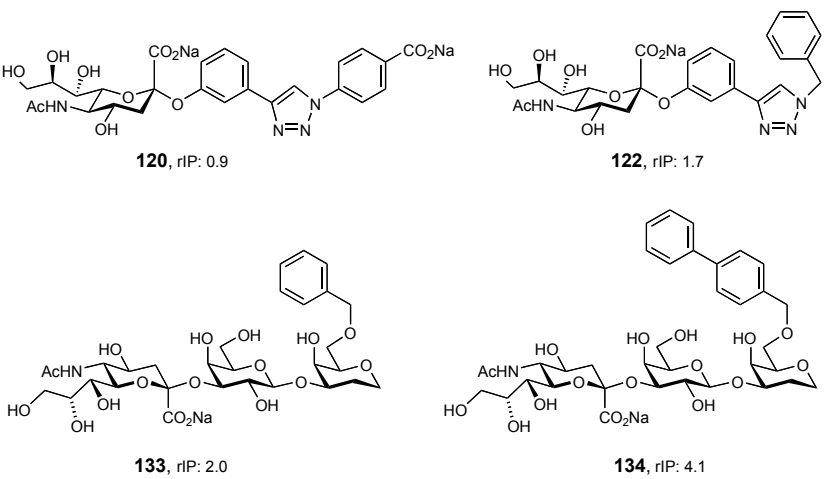
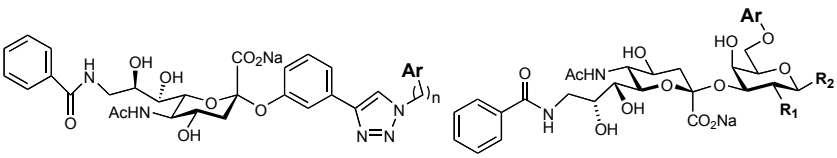


Figure 21: Mimics with hydrophobic substitutions of the α2,6-linked sialic acid moiety.

Based on these observations, mimics **135**-**137** were designed to explore the potential hydrophobic binding site on MAG (*figure* 22). Modification at 9-position of the α2,3-linked terminal sialic acid by benzoylamine moiety was kept in all these structures due to the dramatic improvement which was achieved in MAG binding. Mimic **135** (n=1) with one more aromatic functionality can further confirm the hydrophobic interaction, while **135** (n=2, 3) may explore the distance of the potential hydrophobic binding site by different length of linkers.

STD NMR study of tetrasaccharide ligand **86α** indicated the strongest STD effect on 6-position of internal Gal moiety (*figure* 12). Additionally, the homology model showed the hydroxymethyl group, together with the *N*-acetyl group and the glycerol side chain, are involved in the hydrophobic interaction and hydrogen bond network with Tyr65 of MAG (*figure* 13). Therefore, mimic **137** was designed to link the hydrophobic group directly on the Gal or 1,2-dideoxy-Gal moiety, which yielding even simplified structures.



135, n = 1, 2, 3 **136,** R_1 = OH, H; R_2 = OSE, H.

Figure 22: Exploration of the potential hydrophobic binding site on MAG.

Experimental

General Methods

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC, 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. ESI-MS analyses were carried out on a Bruker esquire3000plus_01096 with Bruker Daltonics DataAnalysis 3.0 program. LC/HRMS analyses 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 rt. The mobile phase was CH_3CN/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% CH_3CN in 10 min followed by 8 min at 95% CH_3CN before returning to initial conditions. Elemental analyses were carried out with a Perkin-Elmer 240 analyzer. 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 (MeOH) was dried by refluxing with sodium methoxide and distilled immediately before use. Pyridine (py) was freshly distilled under argon over CaH_2. Tetrahedrofuran (THF) was dried by refluxing with sodium, benzophenone and distilled immediately before use. Dichloromethane (DCM), dichloroethane (DCE), acetonitrile (CH_3CN), and toluene were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). Petrolether (PE) was distilled over $CaCl_2$. Ethanol (EtOH), 2-propanol (*i*-PrOH), ethyl acetate (AcOEt), *N,N'*-dimethylformamide (DMF), carbon tetrachloride (CCl_4) and dioxane from Fluka (puriss) or Merck (absolute grade for analysis) were used without purification. Molecular sieves (3 Å) were activated in vacuum at 500°C for 2 h immediately before use.

Methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosynate (**7**), (GG-003, *I-002, 003*).

By treating Neu5Ac **5** (4.75 g, 15.0 mmol) with *cat*. Amberlyst 15 in MeOH (250 mL) overnight, methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosylonate (**6**) was achieved (4.50 g, 93%) as a white solid. Without further purification, **6** (1.00 g, 3.00 mmol) was stirred with Ac_2O (13.5 mL, 0.132 mol), py (12.0 mL, 0.152 mol) and *cat*. DMAP at rt for 48 h under argon. The mixture was concentrated under reduced pressure, co-evaporated with toluene (3×20 mL) and the residue was purified by silica gel chromatography (DCM/MeOH 40:1) to afford **7** (1.50 g, 91%) as white foam.[134]

Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate (**8**), (GG-005, *I-005*).

In a sealed vessel (Bombenrohr), AcCl (3.40 mL, 47.6 mmol) and *conc*. HCl (0.426 mL, 4.23 mmol) were subsequently added to a stirred solution of **7** (1.50 g, 2.80 mmol) in DCM (10 mL) at –20°C. After stirring at rt for 16 h, the mixture was concentrated and the residue was diluted with DCM (50 mL), and washed with H_2O (2×30 mL), 5% $KHCO_3$ (2×30 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford **8** (1.11 g, 77%) as white foam, which was used in the next step without further purification.

***O*-Ethyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)dithiocarbonate** (**2**), (GG-007, *I-007*).

A solution of **8** (1.11 g, 2.20 mmol), potassium ethyl xanthogenate (0.423 g, 2.64 mmol), and TBAHS (0.740 g, 2.20 mmol) in AcOEt (12 mL) and 5% $NaHCO_3$ (12 mL) was stirred at rt for 3 h. The mixture was diluted with AcOEt (30 mL), washed with H_2O (2×30 mL) and brine (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/AcOEt/EtOH 6:4:1) to afford **2** (0.670 g, 51%) as white foam, the NMR data of which were in accordance with Lit.[133]

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyransyl bromide (**11**), (GG-004, *I-004*).

Per-acetylated galactose **10** (10.0 g, 25.6 mmol) was dissolved in DCM (45.0 mL). 30% HBr in AcOH (90 mL, 0.512 mol) was added dropwise within 2 h at 0°C. The reaction was stirred at 0°C for additional 2 h, and poured onto icewater (250 mL), which was extracted with DCM (3×80 mL). The combined organic layers were washed with 5% $NaHCO_3$ (3×60 mL), brine (2×60 mL) and dried over Na_2SO_4. After filtration and concentration under reduced pressure, **11** (9.17g, 87%) was obtained as white foam, which was used in the next step without further purification.

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**12**), (GG-006, *I-006*).

A suspension of **11** (9.17 g, 22.3 mmol), HgO (4.83 g, 22.3 mmol), $HgBr_2$ (61.0 mg, 0.170 mmol), $CaSO_4$ (6.30 g, 44.6 mmol) and 2-(trimethylsilyl)ethanol (4.80 mL, 33.5 mmol) in toluene (200 mL) was stirred with light exclusion at rt for 12 h. The mixture was filtered through a pad of Celite, and the Celite was washed with toluene (3×20 mL). The combined filtrates were concentrated under reduced pressure and the residue was purified by silica gel chromatography (PE/AcOEt 2:1) to afford **12** (9.10 g, 91%) as white foam, the NMR data of which were in accordance with Lit.[131]

2-(Trimethylsilyl)ethyl β-D-galactopyranoside (**13**), (GG-008, *I-008*).

To a stirred solution of **12** (9.10 g, 20.3 mmol) in MeOH (50 mL) was added freshly prepared 1M NaOMe/MeOH (5.0 mL). The mixture was stirred at rt under argon for 2 h, then neutralized with Amberlite IRC 50 ion-exchange resin and filtered through a pad of Celite. The Celite was washed with MeOH (3×30 mL). The combined filtrates were concentrated under reduced pressure to afford **13** (5.59 g, 98%) as white foam, which was used in the next step without further purification.

2-(Trimethylsilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside (**3**), (GG-009, *I-009*).

To a stirred solution of **13** (5.59 g, 20.0 mmol) in Et_3N (30 mL) and CH_3CN (120 mL) at –40°C under argon, benzoyl cyanide (3.15 g, 24.0 mmol) was added. The reaction was stirred at –30°C for 2 h and then quenched by adding MeOH (10 mL). The solution was concentrated under reduced pressure, and the residue was then dissolved in DCM (80 mL), and washed successively with 2M HCl (2×30 mL), 5% $NaHCO_3$ (2×30 mL), H_2O (2×30 mL) and brine (30 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/EtOH 8:1) to afford **3** (4.29 g, 56%) as white foam, the NMR data of which were in accordance with Lit.[137]

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-galactopyranoside (**14**), (GG-010, *I-010*).

To a stirred solution of **2** (300 mg, 0.500 mmol) and **3** (290 mg, 0.755 mmol) in CH_3CN/DCM (3:2, 20 mL) was added activated powdered molecular sieves 3 Å (850 mg). The suspension was stirred at rt under argon for 5 h and then cooled to –70°C. After the addition of NIS (215 mg, 0.960 mmol) and TfOH (8.3 μL, 96.0 μmol), stirring was continued for 16 h at –70°C. Then the mixture was diluted with DCM (20 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3×20 mL). The combined filtrates were successively washed with 20% $Na_2S_2O_3$ (30 mL), saturated $KHCO_3$ (2×20 mL) and H_2O (20 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (DCM/*i*-PrOH 20:1) to afford **14** (123 mg, 29%) as white foam, the NMR data of which were in accordance with Lit.[137]

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-galactopyranoside (**15**), (GG-014, *I-014*).

To a stirred solution of **14** (200 mg, 0.233 mmol) at 0°C in py (5.0 mL) was added Ac_2O (3.0 mL). The mixture was stirred for 16 h at rt under argon, and then quenched by adding MeOH (2 mL). The solution was concentrated under reduced pressure and co-evaporated with toluene (3×10 mL). The resulting syrup was purified by silica gel chromatography (DCM/MeOH 30:1) to afford **15** (140 mg, 64%) as white foam, the NMR data of which were in accordance with Lit.[137]

***O*-(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-D-galactopyranoside** (**16**), (GG-018, *I-018*).

To a stirred solution of **15** (140 mg, 0.149 mmol) in DCM (4.0 mL) at –10°C was added $BF_3{\cdot}Et_2O$ (0.1 mL). The mixture was stirred at 0°C for 6 h, then diluted with DCM (10 mL), washed with 5% $NaHCO_3$ (2×5 mL), brine (5 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (DCM/MeOH 30:1) to afford **16** (105 mg, 84%) as white foam, the NMR data of which were in accordance with Lit.[137]

***O*-(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 trichloroacetimidate** (**17**), (GG-2-018, *II-018*).

To a stirred solution of **16** (170 mg, 0.200 mmol) in DCM (5 mL) at -5°C were added CCl_3CN (607 μL, 6.06 mmol) and Cs_2CO_3 (7.24 mg, 0.022 mmol). The mixture was stirred at 0°C for 4 h, and then concentrated. The residue was purified by silica gel chromatography (PE/AcOEt, gradient 1:1 to 1:10) to afford **17** (145 mg, 74%) as white foam.

$[\alpha]_D$ –4.9°, (*c* = 1.35, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.74 (t, $J_{3a,3b} = J_{3a,4} =$ 12.4 Hz, 1H, Sia-H3a), 1.85 (s, 3H, CH_3-NHAc), 2.01, 2.04, 2.07, 2.14, 2.15, 2.18 (6s, 18H, 6×CH_3-OAc), 2.61 (dd, $J_{3a,3b} =$ 12.7, $J_{3b,4} =$ 4.7 Hz, 1H, Sia-H3b), 3.64 (dd, $J_{5,6} =$ 10.7, $J_{6,7} =$ 2.7 Hz, 1H, Sia-H6), 3.78 (s, 3H, OCH_3), 3.98 (dd, $J_{8,9a} =$ 5.9, $J_{9a,9b} =$ 12.5 Hz, 1H, Sia-H9a), 4.06 (q, $J_{4,5} = J_{5,6} = J_{5,NH} =$ 10.4 Hz, 1H, Sia-H5), 4.21-4.28 (m, 2H, Gal-H5, Gal-H6a), 4.40 (dd, $J_{8,9b} =$ 2.5, $J_{9a,9b} =$ 12.5 Hz, 1H, Sia-H9b), 4.45 (dd, $J_{5,6b} =$ 5.6, $J_{6a,6b} =$ 10.3 Hz, 1H, Gal-H6b), 4.79 (dd, $J_{2,3} =$ 10.2, $J_{3,4} =$ 3.4 Hz, 1H, Gal-H3), 4.89 (ddd, $J_{3a,b} =$ 12.1, $J_{3b,4} =$ 4.7, $J_{4,5} =$ 10.5 Hz, 1H, Sia-H4), 5.10 (d, $J_{5,NH} =$ 10.3 Hz, 1H, Sia-NH), 5.13 (d, $J_{3,4} =$ 3.3 Hz, 1H, Gal-H4), 5.33 (dd, $J_{1,2} =$ 8.3, $J_{2,3} =$ 10.2 Hz, 1H, Gal-H2), 5.37 (dd, $J_{6,7} =$ 2.7, $J_{7,8} =$ 9.0 Hz, 1H, Sia-H7), 5.57 (ddd, $J_{7,8} =$ 8.7, $J_{8,9a} =$ 5.9, $J_{8,9b} =$ 2.5 Hz, 1H, Sia-H8), 5.99 (d, $J_{1,2} =$ 8.3 Hz, 1H, Gal-H1), 7.39-7.46, 7.54-7.58, 8.01-8.03 (m, 5H, C_6H_5), 8.68 (s, 1H, NH);

^{13}C NMR (125 MHz, $CDCl_3$): δ 21.55, 21.61, 21.62, 22.00, 22.27 (6C, 6×CH_3-OAc), 24.01 (CH_3-NHAc), 38.31 (Sia-C3), 49.95 (Sia-C5), 53.95 (OCH_3), 62.33 (Gal-C6), 63.37 (Sia-C9), 67.97 (Sia-C7), 68.18 (Gal-C4), 68.89 (Sia-C8), 69.63 (Gal-C2), 70.14 (Sia-C4), 72.10 (Gal-C3), 72.29 (Gal-C5), 73.01 (Sia-C6), 91.49 (Gal-C1), 97.03, 97.69 ($\underline{C}Cl_3$, Sia-C2), 129.15, 130.59, 134.01 (4C, C_6H_5-$\underline{C}$H), 161.95, 166.59,

168.77, 170.52, 171.09, 171.22, 171.42, 171.48, 171.73 (11C, C=NH, C_6H_5-C, 9×CO);

ESI-MS: Calcd for $C_{39}H_{47}C_{l3}N_2O_{21}$ $(M+Na)^+$: 1007.2, 1009.2; Found: m/z 1007.0, 1009.0.

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)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (**18**), (GG-2-024-3, *II-024*).

To a solution of **17** (68.1 mg, 69.0 μmol) and **4** (28.3 mg, 69.0 μmol) in DCM (3 mL) was added activated powdered molecular sieves 3Å (600 mg). The mixture was stirred at rt under argon for 1 h, then cooled to 0°C. To the stirred mixture was added TMSOTf (3.74 μL, 21.0 μmol), and the stirring was continued for 4 h at 0°C. The reaction was quenched by adding a few drops of Et_3N, then diluted with DCM (5 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3×5 mL) and the combined filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (0.5% gradient of MeOH in DCM) to afford **19** (38.7 mg, 46%) and **18** (11.0mg, 13%) as white foams.

19: $[\alpha]_D$ +0.65°, (*c* = 0.77, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.89 (m, 1H, $SiCH_2$-Ha), 0.99 (m, 1H, $SiCH_2$-Hb), 1.73 (s, 3H, CH_3CO_3), 1.87 (s, 3H, CH_3-NHAc), 1.88 (m, 1H, Sia-H3a), 1.94 (s, 3H, CH_3-NHAc), 1.97, 1.98, 2.01, 2.03, 2.08 (5s, 15H, 5×CH_3-OAc), 2.63 (dd, $J_{3a,3b}$ = 12.7, $J_{3b,4}$ = 4.7 Hz, 1H, Sia-H3b), 3.17 (m, 1H, Gal*N*Ac-H2), 3.52 (s, 1H, Gal*N*Ac-H5), 3.56 (m, 1H, OCH_2-Ha), 3.77 (s, 3H, OCH_3), 3.83 (dd, $J_{5,6}$ = 10.8, $J_{6,7}$ = 2.0 Hz, 1H, Sia-H6), 3.98-4.05 (m, 2H, Sia-H5, OCH_2-Hb), 4.08-4.15 (m, 3H, Gal*N*Ac-H6a, Sia-H9a, Sia-H9b), 4.17 (dd, $J_{5,6a}$ = 6.5, $J_{6a,6b}$ = 11.2 Hz, 1H, Gal-H6a), 4.32-4.34 (m, 3H, Gal-H2, Gal-H5, Gal*N*Ac-H6b), 4.40-4.44 (m, 2H, Gal-H6b, Gal*N*Ac-H4), 4.57 (m, 1H, Gal-H3), 4.73 (dd, $J_{2,3}$ = 10.8, $J_{3,4}$ = 3.8 Hz, 1H, Gal*N*Ac-H3), 4.93 (ddd, $J_{3a,4}$ = 12.0, $J_{3b,4}$ = 4.7, $J_{4,5}$ = 10.5 Hz, 1H, Sia-H4), 5.17 (d, $J_{5,NH}$ = 10.0 Hz, 1H, Sia-NH), 5.20 (dd. $J_{3,4}$ = 4.0, $J_{4,5}$ = 1.9 Hz, 1H, Gal-H4), 5.29-5.32 (m, 2H, Gal*N*Ac-H1, Sia-H7), 5.55 (m, 1H, Sia-H8), 5.59 (s, 1H, PhCH), 5.72 (d, $J_{1,2}$ = 5.2 Hz, 1H, Gal-H1), 6.17 (d, $J_{2,NH}$ = 6.7 Hz, 1H, Gal*N*Ac-NH), 7.33-7.38, 7.41-7.44, 7.51-7.57, 8.03-8.05 (m, 10H, 2×C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.41 (3C, $SiMe_3$), 17.92 ($SiCH_2$), 20.50, 20.63, 20.69, 20.70, 20.81 (5×CH_3-OAc), 22.19 ($\underline{C}H_3CO_3$), 23.20, 23.70 (2× CH_3-NHAc), 38.06 (Sia-C3), 49.34 (Sia-C5), 53.12 (OCH_3), 55.07 (Gal*N*Ac-C2), 62.09 (Gal-C6), 62.18 (Sia-C9), 65.94 (Gal-C4), 66.35 (Gal*N*Ac-C5), 66.80 (OCH_2), 66.91 (Sia-C7), 68.06 (Sia-C8), 68.94 (Gal-C2), 68.99 (Sia-C4), 69.09 (Gal*N*Ac-C3), 69.38 (Gal*N*Ac-C6), 70.86 (Gal-C3), 72.21 (Sia-C6), 75.41 (Gal*N*Ac-C4), 75.73 (Gal-C5), 97.35 (Sia-C2), 97.80 (Gal-C1), 98.14 (Gal*N*Ac-C1), 100.75 (Ph$\underline{C}$H), 121.05 ($\underline{C}O_3$), 126.22, 126.42, 128.07, 128.31, 128.91, 129.70, 129.75, 133.16, 138.00 (12C, 2×C_6H_5), 165.912, 167.96, 169.83, 170.02, 170.13, 170.27, 170.83, 170.93, 171.12 (9×CO);

ESI-MS: Calcd for $C_{57}H_{76}N_2O_{26}Si$ $(M+Na)^+$: 1255.4; Found: m/z 1255.1;

18: $[\alpha]_D$ +8.8°, (c = 0.73, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.84-1.00 (m, 2H, $SiCH_2$), 1.71 (m, 1H, Sia-H3a), 1.86, 1.98 (2s, 6H, 2×CH_3-NHAc), 2.01, 2.07, 2.09, 2.12, 2.14, 2.22 (6s, 18H, 6×CH_3-OAc), 2.59 (dd, $J_{3a,3b}$ = 12.7, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.32 (s, 1H, Gal*N*Ac-H5), 3.47-3.56 (m, 2H, Gal*N*Ac-H2, OCH_2-Ha), 3.63 (dd, $J_{5,6}$ = 10.7, $J_{6,7}$ = 2.5 Hz, 1H, Sia-H6), 3.79 (s, 3H, OCH_3), 3.82 (m, 1H, Gal*N*Ac-H6a), 3.96-4.09 (m, 4H, Gal-H5, Sia-H5, Sia-H9a, OCH_2-Hb), 4.16 (dd, $J_{5,6a}$ = 5.9, $J_{6a,6b}$ = 11.1 Hz, 1H, Gal-H6a), 4.21 (d, $J_{6a,6b}$ = 11.8 Hz, 1H, Gal*N*Ac-H6b), 4.28 (d, $J_{3,4}$ = 2.9 Hz, 1H, Gal*N*Ac-H4), 4.34 (dd, $J_{8,9b}$ = 2.7, $J_{9a,9b}$ = 12.5 Hz, Sia-H9b), 4.45 (dd, $J_{5,6b}$ = 6.9, $J_{6a,6b}$ = 11.1 Hz, 1H, Gal-H6b), 4.58-4.63 (m, 2H, Gal-H3, Gal*N*Ac-H3), 4.83 (d, $J_{1,2}$ = 7.9 Hz, 1H, Gal-H1), 4.89 (td, $J_{3a,4}$ = $J_{4,5}$ = 11.9, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H4), 5.04 (d. $J_{3,4}$ = 3.1Hz, 1H, Gal-H4), 5.09-5.14 (m, 3H, Gal-H2, Gal*N*Ac-H1, Sia-NH), 5.39 (dd, 1H, $J_{6,7}$ = 2.5, $J_{7,8}$ = 9.3 Hz, Sia-H7), 5.45 (s, 1H, PhC$\underline{H}$), 5.53 (m, 1H, Sia-H8), 5.74 (d, $J_{2,\ NH}$ = 7.1 Hz, 1H, Gal*N*Ac-NH), 7.15-7.18, 7.24-7.27, 7.28-7.38, 7.42-7.61, 8.01-8.07 (m, 10H, 2×C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.40 (3C, $SiMe_3$), 17.95 ($SiCH_2$), 20.76, 20.78, 20.86, 21.10, 21.40 (6C, 6×CH_3-OAc), 23.16, 23.62 (2× CH_3-NHAc), 37.38 (Sia-C3), 49.14 (Sia-C5), 53.14 (OCH_3), 54.25 (Gal*N*Ac-C2), 62.23 (Sia-C9), 62.42 (Gal-C6), 66.40 (Gal*N*Ac-C5), 66.64 (OCH_2), 66.99 (Sia-C7), 67.49 (Gal-C4), 67.66 (Sia-C8), 69.24 (Gal*N*Ac-C6), 69.26 (Sia-C4), 70.05 (Gal-C2), 70.56 (Gal-C5), 71.68 (Gal-C3), 72.09 (Sia-C6), 75.14 (Gal*N*Ac-C4), 75.99 (Gal*N*Ac-C3), 96.74 (Sia-C2), 98.59 (Gal*N*Ac-C1), 100.76 (Ph$\underline{C}$H), 101.17 (Gal-C1), 126.31, 127.98, 128.20, 128.42, 128.67, 129.01, 129.63, 133.33 (12C, 2×C_6H_5), 165.80, 167.96, 169.49, 170.45, 170.58, 170.70, 170.89 (10C, 10×CO);

ESI-MS: Calcd for $C_{57}H_{76}N_2O_{26}Si$ $(M+Na)^+$: 1255.4; Found: m/z 1255.0.

2-(Trimethylsilyl)ethyl (sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-(β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside (**1**), (GG-2-026, *II-026*).

A solution of **18** (14.0 mg, 11.4 μmol) in 80% aq. AcOH (2.0 ml) was stirred for 4 h at 45°C. The solvent was evaporated in vacua. The remaining solid was dissolved in MeOH (1.8 mL) and treated with freshly prepared 1 M NaOMe/MeOH (0.2 mL). The mixture was stirred at rt under argon for 4 h, then H_2O (0.25 mL) was added and the mixture was stirred for 16 h at rt. The solution was concentrated and the residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **1** as white solid (4.5 mg, 50%) after a final lyophilization from H_2O.

$[\alpha]_D$ –7.8°, (*c* = 0.25, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.00 (s, 9H, $SiMe_3$), 0.86 (m, 1H, $SiCH_2$-Ha), 0.96 (m, 1H, $SiCH_2$-Hb), 1.78 (t, $J_{3a,3b} = J_{3a,4} = 11.6$ Hz, 1H, Sia-H3a), 2.00, 2.02 (2s, 6H, 2×CH_3-NHAc), 2.74 (m, 1H, Sia-H3b), 3.54 (m, 1H, Gal-H2), 3.57-3.62 (m, 3H, Gal*N*Ac-H5, Sia-H6, Sia-H7), 3.63-3.74 (m, 5H, Gal*N*Ac-H6, Gal-H6a, Sia-H4, Sia-H9a, OCH_2-Ha), 3.75-3.81 (m, 2H, Gal-H5, Gal-H6b), 3.82-3.87 (m, 4H, Gal*N*Ac-H3, Sia-H5, Sia-H8, Sia-H9b), 3.92 (m, 1H, Gal-H4), 3.97-4.06 (m, 3H, Gal*N*Ac-H2, Gal*N*Ac-H3, OCH_2-Hb), 4.16 (m, 1H, Gal*N*Ac-H4), 4.49 (d, $J_{1,2}$ = 7.6 Hz, 1H, Gal-H1), 4.53 (d, $J_{1,2}$ = 8.3 Hz, 1H, Gal*N*Ac-H1);

^{13}C NMR (125 MHz, D_2O): δ -1.8 (3C, $SiMe_3$), 17.6 ($SiCH_2$), 22.4, 22.6 (2×CH_3-NHAc), 40.1 (Sia-C3), 51.6 (Gal*N*Ac-C2), 52.2 (Sia-C5), 61.3, 61.4 (Gal*N*Ac-C6, Gal-C6), 62.7 (Sia-C9), 67.7 (Gal-C4), 68.2 (Gal*N*Ac-C4), 68.3 (Sia-C7), 68.5 (Sia-C4), 68.6 (OCH_2), 69.2 (Gal-C2), 72.2 (Sia-C8), 72.7 (Sia-C6), 75.0, 75.1 (Gal*N*Ac-C5, Gal-C-5), 76.1 (Gal-C3), 80.8 (Gal*N*Ac-C3), 100.2 (Sia-C2), 100.8 (Gal*N*Ac-C1), 104.9 (Gal-C1);

HRMS (FAB) Calcd for $C_{30}H_{54}N_2O_{19}Si$ $(M+H)^+$: 775.3168; Found: m/z 775.3110.

2-(Trimethylsilyl)ethyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**23**), (GG-037, *I-037*).

To a stirred solution of **13** (170 mg, 0.606 mmol) and α,α-dimethoxytoluene (0.167 mL, 1.21 mmol) in CH_3CN (3 mL) at 0°C, $TsOH{\cdot}H_2O$ (8.80 mg, 46.0 μmol) was

added. The mixture was stirred at rt for 2.5 h, and then quenched by adding a few drops of Et_3N. After concentration under reduced pressure, the residue was purified by silica gel chromatography (PE/AcOEt 3:2) to afford **23** (200 mg, 90%) as white foam, the NMR data of which were in accordance with Lit.[131]

2-(Trimethylsilyl)ethyl (2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-β-D-galactopyranoside (**25**) and **2-(Trimethylsilyl)ethyl (2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-galactopyranoside** (**26**), (GG-040-2 and GG-040-3, *I-040*).

To a stirred solution of **23** (50.0 mg, 0.136 mmol) and ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside[145] **24** (102 g, 0.163 mmol) in DCM (3.5 mL) was added activated powdered molecular sieves 3 Å (200 mg). The reaction mixture was stirred at rt under argon for 2 h and then cooled to –30°C. After the addition of NIS (73.0 mg, 0.326 mmol) and TfOH (3.0 μL, 32.6 μmol), stirring was continued for 1.5 h at –30°C. Then the reaction was quenched by adding a few drops of Et_3N, diluted with DCM (10 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3×5 mL), the combined filtrates were successively washed with 20% $Na_2S_2O_3$ (15 mL), saturated aq. $KHCO_3$ (2×10 mL) and H_2O (10 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (PE/AcOEt gradient 5:1 to 3:1) to afford **25** (17.0 mg, 13%) and **26** (50.0 mg, 40%) as white foams.

25: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.95-1.06 (m, 2H, $SiCH_2$), 3.37 (bs, 1H, Gal-H5), 3.55 (m, 1H, OCH_2-Ha), 3.66 (dd, $J_{5,6a}$ = 6.0, $J_{6a,6b}$ = 9.7 Hz, 1H, Gal'-H6a), 3.71 (dd, $J_{5,6b}$ = 6.7, $J_{6a,6b}$ = 9.7 Hz, 1H, Gal'-H6b), 3.81 (dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 3.5 Hz, 1H, Gal-H3), 3.92 (dd, $J_{5,6a}$ = 1.6, $J_{6a,6b}$ = 12.4 Hz, 1H, Gal-H6a), 3.93 (m, 1H, Gal-H2), 4.00 (m, 1H, OCH_2-Hb), 4.18 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, 1H, Gal'-H5) 4.26 (d, $J_{3,4}$ = 3.8 Hz, 1H, Gal-H4), 4.28 (m, 1H, Gal-H6b), 4.32 (d, $J_{1,2}$ = 7.5 Hz, 1H, Gal-H1), 4.44, 4.50 (A, B of AB, J = 11.8 Hz, 1H, PhC$\underline{H}_2$), 5.34(d, $J_{1,2}$ = 8.0 Hz, 1H, Gal'-H1), 5.41 (s, 1H, PhC$\underline{H}$), 5.56 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.5 Hz, 1H, Gal'-H3), 5.80 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.4 Hz, 1H, Gal'-H2), 5.92 (d, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H4), 7.18-7.26, 7.30-7.35, 7.39-7.51, 7.59-7.62, 7.78-7.80, 7.91-7.93, 8.04-8.05 (m, 25H, 5×C_6H_5);

26: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.04 (s, 9H, $SiMe_3$), 1.03-1.08 (m, 2H, $SiCH_2$), 2.30 (d, $J_{3,OH}$ = 7.4 Hz, 1H, Gal-3-OH), 3.39 (bs, 1H, Gal-H5), 3.59-3.72 (m, 4H, Gal-

H3, Gal'-H6a, Gal'-H6b, OCH_2-Ha), 3.81 (dd, $J_{1,2}$ = 7.7, $J_{2,3}$ = 9.4 Hz, 1H, Gal-H2), 4.00 (dd, $J_{5,6a}$ = 1.7, $J_{6a,6b}$ = 12.3 Hz, 1H, Gal-H6a), 4.04 (m, 1H, OCH_2-Hb), 4.07 (d, $J_{3,4}$ = 3.6 Hz, 1H, Gal-H4), 4.17 (m, 1H, Gal'-H5), 4.30 (m, 1H, Gal-H6b), 4.37, 4.51 (A, B of AB, J = 11.8 Hz, 2H, PhC$\underline{H}_2$), 4.44 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal-H1), 5.28(d, $J_{1,2}$ = 8.1 Hz, 1H, Gal'-H1), 5.43 (s, 1H, PhC$\underline{H}$), 5.53 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H3), 5.76 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.4 Hz, 1H, Gal'-H2), 5.97 (d, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H4), 7.13-7.31, 7.32-7.45, 7.58-7.61, 7.78-7.79, 7.91-7.93, 8.02-8.04 (m, 25H, 5×C_6H_5).

2-(Trimethylsilyl)ethyl 3-*O*-benzyl- 4,6-*O*-benzylidene-β-D-galactopyranoside (27), **2-(trimethylsilyl)ethyl 2-*O*-benzyl- 4,6-*O*-benzylidene-β-D-galactopyranoside** (**28**) and **2-(trimethylsilyl)ethyl 2,3-di-*O*-benzyl- 4,6-*O*-benzylidene-β-D-galactopyranoside** (**29**), (GG-042-2, GG-042-3, and GG-042-1, *I-042*).

To a stirred solution of **23** (150 mg, 0.410 mmol), TBAB (27.0 mg, 82.0 μmol), and benzyl bromide (85.0 μL, 0.700 mmol) in toluene (7.5 mL) was added aq. 5% NaOH (0.75 mL). The mixture was stirred vigorously at 60°C for 22 h. The solution was diluted with toluene (10 mL), the organic layer was washed with H_2O (2×20 mL) and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (PE/AcOEt 2:1) to afford **27** (118 mg, 63%), **28** (25.2 mg, 13%) and **29** (10.0 mg, 4%) as white foams.

27: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (s, 9H, $SiMe_3$), 0.91-1.09 (m, 2H, $SiCH_2$), 2.43 (bs, 1H, 2-OH), 3.36 (bs, 1H, Gal-H5), 3.50 (dd, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.6 Hz, 1H, Gal-H3), 3.57 (m, 1H, OCH_2-Ha), 3.97-4.08 (m, 3H, Gal-H2, Gal-H6a, OCH_2-Hb), 4.13 (d, $J_{3,4}$ = 3.5 Hz, 1H, Gal-H4), 4.31 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal-H1), 4.32 (m, 1H, Gal-H6b), 4.75, 4.78 (A, B of AB, J = 12.4 Hz, 2H, PhC$\underline{H}_2$), 5.46 (s, 1H, PhC$\underline{H}$), 7.28-7.41, 7.51-7.54 (m, 10H, 2×C_6H_5);

28: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.03 (s, 9H, $SiMe_3$), 0.99-1.12 (m, 2H, $SiCH_2$), 2.49 (d, $J_{3,OH}$ = 7.4 Hz, 1H, 3-OH), 3.45 (bs, 1H, Gal-H5), 3.58 (m, 1H, OCH_2-Ha), 3.62 (dd, $J_{1,2}$ = 7.7, $J_{2,3}$ = 9.4 Hz, 1H, Gal-H2), 3.74 (m, 1H, Gal-H3), 4.04-4.10 (m, 2H, Gal-H6a, OCH_2-Hb), 4.22 (d, $J_{3,4}$ = 3.8 Hz, 1H, Gal-H4), 4.34 (dd, $J_{5,6a}$ = 1.3, $J_{6a,6b}$ = 12.4 Hz, 1H, Gal-H6b), 4.41 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal-H1), 4.73, 5.00 (A, B of AB,

J = 11.3 Hz, 2H, PhC$\underline{H}_2$), 5.56 (s, 1H, PhC$\underline{H}$), 7.26-7.29, 7.32-7.40, 7.51-7.53 (m, 10H, 2×C_6H_5);

29: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (s, 9H, $SiMe_3$), 0.98-1.08 (m, 2H, $SiCH_2$), 3.31 (bs, 1H, Gal-H5), 3.55 (dd, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.7 Hz, 1H, Gal-H3), 3.58 (m, 1H, OCH_2-Ha), 3.84 (dd, $J_{1,2}$ = 7.8, $J_{2,3}$ = 9.7 Hz, 1H, Gal-H2), 4.02 (dd, $J_{5,6a}$ = 1.7, $J_{6a,6b}$ = 12.3 Hz, 1H, Gal-H6a), 4.06 (m, 1H, OCH_2-Hb), 4.11 (d, $J_{3,4}$ = 3.4 Hz, 1H, Gal-H4), 4.31 (dd, $J_{5,6a}$ = 1.4, $J_{6a,6b}$ = 12.3 Hz, 1H, Gal-H6b), 4.39 (d, $J_{1,2}$ = 7.8 Hz, 1H, Gal-H1), 4.73-4.80 (m, 3H, PhC$\underline{H}_2$, PhC$\underline{H}_2$-HA), 4.95 (B of AB, *J* = 10.9 Hz, PhC$\underline{H}_2$-HB), 5.49 (s, 1H, PhC$\underline{H}$), 7.26-7.40, 7.55-7.56 (m, 15H, 3×C_6H_5).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-6-*O*-benzoyl-β-D-galactopyranoside (**31**) and **2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→2)-6-*O*-benzoyl-β-D-galactopyranoside** (**32**), (GG-048-1 and GG-048-2, *I-048*).

To a stirred solution of **3** (30.0 mg, 78.0 μmol) and ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside[146] **30** (55.0 g, 86.0 μmol) in DCM (2.0 mL) was added activated powdered molecular sieves 3 Å (150 mg). The suspension was stirred at rt under argon for 2 h and then cooled to –30°C. After the addition of NIS (38.7 mg, 0.172 mmol) and TfOH (1.5 μL, 17.2 μmol), stirring was continued for 3 h at –30°C. Then the reaction was quenched by adding few drops of Et_3N, 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 successively washed with 20% $Na_2S_2O_3$ (15 mL), saturated $KHCO_3$ (2×10 mL), H_2O (10 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (PE/AcOEt 4:1 to 2:1) to afford **31** (7.00 mg, 9%) and **32** (20.0 mg, 27%) as white foams.

31: ^{1}H NMR (500 MHz, $CDCl_3$): δ -0.05 (s, 9H, $SiMe_3$), 0.91-0.99 (m, 2H, $SiCH_2$), 3.53 (m, 1H, OCH_2-Ha), 3.69-3.76 (m, 3H, Gal-H2, Gal-H3, Gal-H5), 3.95 (m, 1H, OCH_2-Hb), 4.11 (d, $J_{3,4}$ = 3.0 Hz, 1H, Gal-H4), 4.20 (d, $J_{1,2}$ = 7.3 Hz, 1H, Gal-H1), 4.37 (m, 1H, Gal'-H5), 4.42-4.48 (m, 2H, Gal-H6a, Gal'-H6a), 4.55 (dd, $J_{5,6b}$ = 7.6, $J_{6a,6b}$ = 11.5 Hz, 1H, Gal-H6b), 4.65 (dd, $J_{5,6b}$ = 7.1, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal'-H6b), 5.19 (d, $J_{1,2}$ = 7.9 Hz, 1H, Gal'-H1), 5.66 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 3.5 Hz, 1H, Gal'-H3), 5.79 (dd, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.4 Hz, 1H, Gal'-H2), 5.99 (d, $J_{3,4}$ = 3.5 Hz, 1H, Gal'-H4),

7.24-7.27, 7.36-7.59, 7.63-7.66, 7.78-7.79, 7.94-7.96, 8.01-8.03, 8.10-8.11 (m, 25H, 5×C_6H_5);

32: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 1.03-1.07 (m, 2H, $SiCH_2$), 3.64 (m, 1H, OCH_2-Ha), 3.71-3.72 (m, 2H, Gal-H2, Gal-H3), 3.78 (m, 1H, Gal-H5), 3.91 (d, $J_{3,4}$ = 1.4 Hz, 1H, Gal-H4), 4.00 (m, 1H, OCH_2-Hb), 4.33-4.40 (m, 2H, Gal'-H5, Gal'-H6a), 4.48 (d, $J_{1,2}$ = 7.4 Hz, 1H, Gal-H1), 4.52 (dd, $J_{5,6a}$ = 7.0, $J_{6a,6b}$ = 11.3 Hz, 1H, Gal-H6a), 4.57 (dd, $J_{5,6b}$ = 6.1, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal-H6b), 4.71 (dd, $J_{5,6b}$ = 9.2, $J_{6a,6b}$ = 14.6 Hz, 1H, Gal'-H6b), 5.30 (d, $J_{1,2}$ = 7.9 Hz, 1H, Gal'-H1), 5.67 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H3), 5.77 (dd, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.5 Hz, 1H, Gal'-H2), 6.01 (d, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H4), 7.22-7.26, 7.37-7.64, 7.76-7.79, 7.95-8.03, 8.09-8.11 (m, 25H, 5×C_6H_5).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranoside (**33**), (GG-048-1', *I-050*).

To a stirred solution of **31** (7.00 mg, 7.27 μmol) in py (0.5 mL) was added Ac_2O (0.25 mL). The mixture was stirred for 16 h at rt under argon, and then quenched by adding MeOH (0.5 mL). The solution was concentrated under reduced pressure and co-evaporated with toluene (3×5 mL). The residue was diluted with DCM (10 mL), washed with 1M HCl (2×10 mL), 5% $NaHCO_3$ (2×10 mL) H_2O (10 mL), and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded **33** as white foam (7.00 mg, 94%).

^{1}H NMR (500 MHz, $CDCl_3$): δ -0.07 (s, 9H, $SiMe_3$), 0.95-0.97 (m, 2H, $SiCH_2$), 2.18, 2.23 (2s, 6H, 2×CH_3-OAc), 3.47 (m, 1H, OCH_2-Ha), 3.87-3.92 (m, 3H, Gal-H3, Gal-H5, OCH_2-Hb), 4.27-4.38 (m, 4H, Gal-H1, Gal-H6a, Gal'-H5, Gal'-H6a), 4.45 (dd, $J_{5,6b}$ = 7.5, $J_{6a,6b}$ = 11.5 Hz, 1H, Gal-H6b), 4.68 (dd, $J_{5,6b}$ = 6.3, $J_{6a,6b}$ = 11.2 Hz, 1H, Gal'-H6b), 4.11 (d, $J_{3,4}$ = 3.0 Hz, 1H, Gal-H4), 4.93 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal'-H1), 5.16 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.0 Hz, 1H, Gal-H2), 5.56 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H3), 5.70 (dd, $J_{1,2}$ = 7.7, $J_{2,3}$ = 10.4 Hz, 1H, Gal'-H2), 5.72 (d, $J_{3,4}$ = 2.8 Hz, 1H, Gal-H4), 5.93 (d, $J_{3,4}$ = 3.2 Hz, 1H, Gal'-H4), 7.22-7.26, 7.35-7.43, 7.48-7.55, 7.62-7.65, 7.75-7.77, 7.91-7.92, 8.00-8.02, 8.05-8.06, 8.13-8.14 (m, 25H, 5×C_6H_5).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→2)-3,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranoside (**34**), (GG-048-2', *I-051*).

To a stirred solution of **32** (20.0 mg, 20.8 μmol) in py (1.0 mL) was added Ac_2O (0.5 mL). The mixture was stirred for 16 h at rt under argon, and then quenched by adding MeOH (1 mL). The solution was concentrated under reduced pressure and co-evaporated with toluene (3×5 mL). The residue was diluted with DCM (10 mL), washed with 1N HCl (2×10 mL), 5% $NaHCO_3$ (2×10 mL), H_2O (10 mL), and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded **34** as white foam (21.0 mg, 98%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.07 (s, 9H, $SiMe_3$), 0.95-0.97 (m, 2H, $SiCH_2$), 2.18, 2.25 (2s, 6H, 2×CH_3-OAc), 3.72 (m, 1H, OCH_2-Ha), 3.95 (dd, 1H, $J_{1,2}$ = 7.8, $J_{2,3}$ = 10.2 Hz, 1H, Gal-H2), 4.00 (m, 1H, Gal-H5), 4.07 (m, 1H, OCH_2-Hb), 4.23 (dd, 1H, $J_{5,6a}$ = 6.9, $J_{6a,6b}$ = 11.2 Hz, 1H, Gal-H6a), 4.29-4.38 (m, 2H, Gal'-H5, Gal'-H6a), 4.50 (dd, $J_{5,6b}$ = 6.8, $J_{6a,6b}$ = 11.2 Hz, 1H, Gal-H6b), 4.62 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal-H1), 4.75 (dd, $J_{5,6b}$ = 2.8, $J_{6a,6b}$ = 8.6 Hz, 1H, Gal'-H6b), 4.99 (dd, $J_{2,3}$ = 10.2, $J_{3,4}$ = 3.4 Hz, 1H, Gal-H3), 5.34 (d, $J_{1,2}$ = 8.0 Hz, 1H, Gal'-H1), 5.39 (d, $J_{3,4}$ = 3.7 Hz, 1H, Gal-H4), 5.53 (dd, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H3), 5.74 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.3 Hz, 1H, Gal'-H2), 5.99 (d, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H4), 7.23-7.26, 7.37-7.44, 7.47-7.63, 7.76-7.78, 7.93-8.00, 8.08-8.10 (m, 25H, 5×C_6H_5).

2-(Trimethylsilyl)ethyl 6-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**35**), (GG-049, *I-049*).

To a refluxing solution of **3** (100 mg, 0.260 mmol) in acetone (3.0 mL) was added TsOH·H_2O (16.5 mg, 87.0 μmol). The mixture was refluxed for 1 h, quenched by adding 5% aq. $NaHCO_3$ till neutral, and then concentrated under reduced pressure. The residue was diluted with DCM (20 mL), washed with H_2O (2×10 mL), and dried over $NaSO_4$. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (PE/AcOEt 3:1) to afford **35** (90.0 mg, 82%) as white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ -0.01 (s, 9H, $SiMe_3$), 0.94-1.07 (m, 2H, $SiCH_2$), 1.37, 1.54 (2s, 6H, 2×CH_3), 2.40 (bs, 1H, 2-OH), 3.55-3.61 (m, 2H, Gal-H2, OCH_2-Ha), 4.00 (m, 1H, OCH_2-Hb), 4.10-4.15 (m, 2H, Gal-H3, Gal-H5), 4.21 (d, $J_{1,2}$ = 8.3 Hz, 1H, Gal-H1), 4.23 (d, $J_{3,4}$ = 5.5, $J_{4,5}$ = 2.2 Hz, 1H, Gal-H4), 4.60 (dd, $J_{5,6a}$ = 7.9, $J_{6a,6b}$ = 11.6 Hz, 1H, Gal-H6a), 4.67 (dd, $J_{5,6b}$ = 4.7, $J_{6a,6b}$ = 11.6 Hz, 1H, Gal-H6b), 7.43-7.46, 7.56-7.60, 8.04-8.06 (m, 5H, C_6H_5).

2-(Trimethylsilyl)ethyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**39**), (GG-065, *I-070*).

To a stirred solution of **13** (1.50 g, 5.35 mmol) in DMF (20 mL) were added 2,2-dimethoxypropane (0.98 mL, 8.02 mmol) and $TsOH \cdot H_2O$ (20.0 mg, 0.107 mmol). The mixture was stirred at 80°C for 3 h, and quenched by adding a few drops of Et_3N. The solution was concentrated under reduced pressure to a syrup, and then purified by silica gel chromatography (PE/AcOEt 1:1) to afford **39** (1.45 mg, 85%) as white foam, the NMR data of which were in accordance with Lit.{Murase, 1989 #20}

2-(Trimethylsilyl)ethyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galacopyranoside (**37**), (GG-067, *I-071*).

To a solution of **39** (1.45 g, 4.52 mmol) in DMF (25 mL) at 0°C were added NaH in (50% suspension in oil, 0.650 g, 13.6 mmol) and benzyl bromide (1.60 mL, 13.6 mmol). The mixture was stirred at 0°C for 1.5 h, then quenched by adding MeOH (2 mL), and evaporated under reduced pressure. The resulting syrup was diluted with DCM (30 mL), washed with H_2O (3×20 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (PE/AcOEt 15:1) to afford **37** (2.00 g, 88%) as white foam, the NMR data of which were in accordance with Lit.[229]

2-(Trimethylsilyl)ethyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (**38**), (GG-068, *I-068*).

A solution of **37** (70.0 mg, 0.139 mmol) in aq. 80% AcOH (2.0 mL) was heated at 60°C for 1 h. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene (3×5 mL). The residue was purified by silica gel chromatography (PE/AcOEt 1:1) to afford **38** (59.0 mg, 92%) as white foam, the NMR data of which were in accordance with Lit.[229]

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-di-*O*-benzyl-β-D-galactopyranoside (**41**), (GG-074, *I-074*).

To a solution of **38** (300 mg, 0.648 mmol) and **30** (500 mg, 0.778 mmol) in DCM (10 mL) was added activated powdered molecular sieves 3Å (2.00 g). The reaction

mixture was stirred at rt for 6 h under argon before DMTST (504 mg, 1.95 mmol) was added at 0°C. The reaction mixture was stirred at 7°C for 16 h, 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 5% $KHCO_3$ (2×20 mL) and H_2O (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (PE/AcOEt 5:1) to afford the corresponding disaccharide **41** (588 mg, 87%) as white foam.

$[\alpha]_D$ +56.4° (*c* = 0.96, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.90-1.02 (m, 2H, $SiCH_2$), 3.54 (ddd, *J* = 5.8, 9.6, 11.5 Hz, 1H, OCH_2-Ha), 3.58-3.62 (m, 2H, Gal-H2, Gal-H5), 3.71 (dd, $J_{5,6a}$ = 5.1, $J_{6a,6b}$ = 10.1 Hz, 1H, Gal-H6a), 3.75 (dd, $J_{5,6b}$ = 6.6, $J_{6a,6b}$ = 10.1 Hz, 1H, Gal-H6b), 3.84 (dd, $J_{2,3}$ = 9.4, $J_{3,4}$ = 3.3 Hz, 1H, Gal-H3), 4.00 (ddd, *J* = 6.1, 9.6, 11.4 Hz, 1H, OCH_2-Hb), 4.13 (d, $J_{3,4}$ =2.8 Hz, 1H, Gal-H4), 4.28 (m, 1H, Gal'-H5), 4.34 (d, $J_{1,2}$ = 7.8 Hz, 1H, Gal-H1), 4.36, 4.72 (A, B of AB, *J* = 11.1 Hz, 2H, PhC$\underline{H}_2$), 4.45 (dd, $J_{5,6a}$ = 6.1, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal'-H6a), 4.55, 4.59 (A, B of AB, *J* = 11.9 Hz, 2H, PhC$\underline{H}_2$), 4.65 (dd, $J_{5,6b}$ = 7.0, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal'-H6b), 5.27(d, $J_{1,2}$ = 8.0 Hz, 1H, Gal'-H1), 5.62 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 3.5 Hz, 1H, Gal'-H3), 5.89 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.5 Hz, 1H, Gal'-H2), 6.00 (dd, $J_{3,4}$ = 3.5, $J_{4,5}$ = 0.8 Hz, 1H, Gal'-H4), 7.16-7.18, 7.25-7.32, 7.35-7.37, 7.43-7.47, 7.53-7.57, 7.58-7.68, 7.78-7.80, 7.87-7.89, 8.03-8.05, 8.12-8.14 (m, 30H, 6×C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.51 (3C, $SiMe_3$), 18.41 ($SiCH_2$), 61.98 (Gal'-C6), 67.25 (OCH_2), 68.09 (Gal'-C4), 68.90 (Gal-C4), 69.49 (Gal-C6), 69.86 (Gal'-C2), 71.42, 71.50 (Gal'-C3, Gal'-C5), 73.16 (Gal-C5), 73.60 (Ph$\underline{C}H_2$), 74.72 (Ph$\underline{C}H_2$), 78.87 (Gal-C2), 80.82 (Gal-C3), 101.62 (Gal'-C1), 102.91 (Gal-C1), 127.46-138.50 (36C, 6×C_6H_5), 165.48, 165.54, 165.60, 165.94 (4×CO);

HRMS (FAB) Calcd for $C_{59}H_{62}O_{15}Si$ $(M+NH_4)^+$: 1056.4202; Found: m/z 1056.4186.

2-(Trimethylsilyl)ethyl (β-D-galactopyranosyl)-(1→3)-2,6-*O*-dibenzyl-β-D-galactopyranoside (**42**), (GG-076, *I-080*).

To a solution of **41** (550 mg, 0.529 mmol) in MeOH (20 mL) was added freshly prepared 1 M NaOMe/MeOH (2.0 mL). The mixture was stirred at rt for 2 h under argon, then neutralized with Amberlite IRC 50 ion-exchange resin and filtered through a pad of Celite. The Celite was washed with MeOH (3×10 mL), and the

combined filtrates were evaporated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH 15:1) to afford **42** (280 mg, 85%) as white foam.

$[\alpha]_D$ -7.7° (*c* = 0.75, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 0.00 (s, 9H, $SiMe_3$), 0.97 (t, *J* = 8.4 Hz, 2H, $SiCH_2$), 3.40-3.44 (m, 2H, Gal'-H3, Gal-H5), 3.54-3.63 (m, 3H, Gal-H2, Gal'-H2, OCH_2-Ha), 3.64-3.71 (m, 5H, Gal-H6a, Gal-H6b, Gal'-H5, Gal'-H6a, Gal'-H6b), 3.76 (dd, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.4 Hz, 1H, Gal-H3), 3.78 (d, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H4), 4.00 (m, 1H, OCH_2-Hb), 4.02 (d, $J_{3,4}$ = 3.4 Hz, 1H, Gal-H4), 4.37 (d, $J_{1,2}$ = 7.8 Hz, 1H, Gal-H1), 4.54, 4.57 (A, B of AB, *J* = 11.9 Hz, 2H, Ph$C\underline{H}_2$), 4.58 (d, $J_{1,2}$ = 7.8 Hz, 1H, Gal'-H1), 4.78, 4.84 (A, B of AB, *J* = 10.7 Hz, 2H, Ph$C\underline{H}_2$), 7.21-7.33, 7.40 (m, 10H, 2×C_6H_5);

^{13}C NMR (125 MHz, CD_3OD): δ -1.49 (3C, $SiMe_3$), 19.28 ($SiCH_2$), 62.41 (Gal'-C6), 68.20 (OCH_2), 70.16 (Gal'-C4), 70.53 (Gal-C4), 70.71 (Gal-C6), 72.65 (Gal'-C3), 74.24 (Ph$\underline{C}H_2$), 74.60 (Gal'-C2), 75.75 (Ph$\underline{C}H_2$), 76.55 (2C, Gal-C5, Gal'-C5), 80.33 (Gal-C2), 81.75 (Gal-C3), 104.33 (Gal-C1), 105.86 (Gal'-C1), 128.39, 128.55, 128.65, 129.02, 129.24, 129.30, 139.56, 140.16 (12C, 12×C_6H_5);

ESI-MS: Calcd for $C_{31}H_{46}O_{11}Si$ $(M+Na)^+$: 645.3; Found: m/z 645.2.

2-(Trimethylsilyl)ethyl (β-D-galactopyranosyl)-(1→3)-β-D-galactopyranoside (**22**), (GG-098, *I-098*).

A solution of **42** (45.0 mg, 72.3 μmol) in MeOH (2.5 mL) containing a catalytic amount of AcOH was hydrogenolyzed (1 bar H_2) in the presence of 10% Pd-C (15.0 mg) for 3 h at rt. The reaction mixture was filtered through a pad of Celite, which was washed with MeOH (5 mL). The filtrate was concentrated and then purified by silica gel chromatography (DCM/MeOH 3:1) to afford **22** (26.6 mg, 83%) as white foam.

$[\alpha]_D$ -2.3° (*c* = 1, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 0.00 (s, 9H, $SiMe_3$), 0.94 (m, 1H, $SiCH_2$-Ha), 1.02 (m, 1H, $SiCH_2$-Hb), 3.46 (dd, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.2 Hz, 1H, Gal'-H3), 3.49-3.52 (m, 2H, Gal-H5, Gal'-H5), 3.55-3.74 (m, 8H, Gal-H2, Gal-H3, Gal-H6a, Gal-H6b, Gal'-H2, Gal'-H6a, Gal'-H6b, OCH_2-Ha), 3.75 (d, $J_{3,4}$ = 3.2 Hz, 1H, Gal'-H4), 3.98 (ddd, *J* = 5.7, 9.6, 11.5Hz, 1H, OCH_2-Hb), 4.08 (d, $J_{3,4}$ = 3.0 Hz, 1H, Gal-H4), 4.26 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal-H1), 4.45 (d, $J_{1,2}$ = 7.6 Hz, 1H, Gal'-H1);

^{13}C NMR (125 MHz, CD_3OD): δ -1.47 (3C, $SiMe_3$), 18.99 ($SiCH_2$), 62.41 (2C, Gal-C6, Gal'-C6), 67.91 (OCH_2), 69.70, 70.13 (Gal-C4, Gal'-C4), 71.43, 72.88 (Gal-C2,

Gal'-C2), 74.52, 76.09 (Gal-C5, Gal'-C5), 76.69 (Gal'-C3), 85.02 (Gal-C3), 103.79 (Gal-C1), 106.29 (Gal'-C1);

HRMS (FAB) Calcd for $C_{17}H_{34}O_{11}Si$ $(M+Na)^+$: 465.1768; Found: m/z 465.1762.

2-(Trimethylsilyl)ethyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (**20**).

22 (10.0 mg, 22.6 μmol) and CMP-sialic acid (22.3 mg, 33.9 μmol) were dissolved in a mixture of 50 mM sodium cacodylate-buffer pH 6.5 (2 mL), 60 mM aqueous $MnCl_2$ (2 mL) and deionized water (1.3 mL) containing BSA (2.30 mg). The mixture was incubated at 37°C with CIAP (2 μL) and recombinant ST3Gal III (500 μL, 9 U/L). After 17 h, additional ST3Gal III (250 μL) was added and the incubation continued for 3 d. The turbid solution was centrifuged and the supernatant passed over a RP-18 column, which was washed with water before the crude product was eluted with MeOH. After evaporation of the solvent, the residue was chromatographied on silica gel (DCM/MeOH/H_2O 10:4:0.4, then 6:4:1) to yield starting material **22** (4.00 mg, 40 %) and trisaccharide **20** (9.70 mg, 59 %) as white solids after a final lyophilization from water.

$[\alpha]_D$ +4.1° (c = 0.63, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.02 (s, 9H, $SiMe_3$), 0.97 (m, 1H, $SiCH_2$-Ha), 1.07 (m, 1H, $SiCH_2$-Hb), 1.79 (t, $J_{3a,3b} = J_{3a,4} = 12.1$ Hz, 1H, Sia-H3a), 2.02 (s, 3H, CH_3-NHAc), 2.75 (dd, $J_{3a,3b} = 12.4$, $J_{3a,4} = 4.5$ Hz, 1H, Sia-H3b), 3.58 (dd, $J_{6,7} = 1.5$, $J_{7,8} = 9.2$ Hz, 1H, Sia-H7), 3.59-3.66 (m, 4H, Gal-H2, Gal'-H2, Sia-H6, Sia-H9a), 3.66-3.77 (m, 8H, Gal-H5, Gal-H6a, Gal-H6b, Gal'-H5, Gal'-H6a, Gal'-H6b, Sia-H4, OCH_2-Ha), 3.80 (dd, $J_{2,3} = 9.9$, $J_{3,4} = 3,4$ Hz, 1H, Gal-H3), 3.84-3.88 (m, 3H, Sia-H5, Sia-H8, Sia-H9b), 3.94 (d, $J_{3,4} = 3.0$ Hz, 1H, Gal'-H4), 4.04 (ddd, J = 5.2, 10.0, 12.7 Hz, 1H, OCH_2-Hb), 4.10 (dd, $J_{2,3} = 9.8$, $J_{3,4} = 3.1$ Hz, 1H, Gal'-H3), 4.18 (d, $J_{3,4} = 3.3$ Hz, 1H, Gal-H4), 4.45 (d, $J_{1,2} = 8.0$ Hz, 1H, Gal-H1), 4.68 (d, $J_{1,2} = 7.8$ Hz, 1H, Gal'-H1);

^{13}C NMR (125 MHz, D_2O): δ -2.0 (3C, $SiMe_3$), 18.1 ($SiCH_2$), 22.6 (CH_3-NHAc), 40.2 (Sia-C3), 52.2 (Sia-C5), 61.4, 61.5 (Gal-C6, Gal'-C6), 63.1 (Sia-C9), 67.9 (Gal-C4), 68.6 (Sia-C7), 68.8 (Sia-C4), 68.9 (OCH_2), 69.0 (Gal-C4), 70.1, 70.3 (Gal-C2, Gal'-C2), 72.4 (Sia-C8), 73.4 (Sia-C6), 75.2, 75.4 (Gal-C5, Gal'-C5), 76.1 (Gal'-C3), 83.1 (Gal-C3), 100.4 (Sia-C2), 102.3 (Gal-C1), 104.6 (Gal'-C1);

HRMS (FAB) Calcd for $C_{28}H_{51}NO_{19}Si$ $(M+NH_4)^+$: 751.3186; Found: m/z 751.3172.

2-(Trimethylsilyl)ethyl (6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-*O*-dibenzyl-β-D-galactopyranoside (**60**), (GG-078, *I-097*).

To a solution of **42** (26.0 mg, 41.6 μmol) in Et_3N (0.5mL) and CH_3CN (2.0mL), a solution of benzoyl cyanide (4.90 mg, 37.4 μmol) in CH_3CN (0.5 mL) was added dropwise at –40°C under argon during 10 min. The reaction was stirred at –40°C for 2h, then quenched with a few drops of MeOH. The solution was concentrated under reduced pressure and the residue was purified by flash silica gel chromatography (DCM/MeOH 40:1) to afford **60** (22.0 mg, 73%) as white foam.

$[\alpha]_D$ -0.30°, (*c* = 0.65, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.00 (s, 9H, $SiMe_3$), 0.94-0.98 (m, 2H, $SiCH_2$), 3.42-3.46 (m, 2H, Gal-H5, Gal-H6a), 3.51 (dd, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H3), 3.55-3.64 (m, 5H, Gal-H2, Gal-H3, Gal-H6b, Gal'-H2, OCH_2-Ha), 3.83 (dd, $J_{5,6a}$ = 4.2, $J_{5,6b}$ = 8.5 Hz, 1H, Gal'-H5), 3.86 (d, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H4), 3.94-3.99 (m, 2H, Gal-H4, OCH_2-Hb), 4.26 (d, $J_{1,2}$ = 7.6 Hz, 1H, Gal-H1), 4.37 (dd, $J_{5,6a}$ = 4.2, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal'-H6a), 4.43, 4.46 (A, B of AB, *J* = 12.0 Hz, 2H, Ph$C\underline{H}_2$), 4.57 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal'-H1), 4.63 (dd, $J_{5,6b}$ = 8.5, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal'-H6b), 4.76 and 4.84 (A, B of AB, *J* = 10.6 Hz, 2H, Ph$C\underline{H}_2$), 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, CD_3OD): δ -1.48 (3C, $SiMe_3$), 19.25 ($SiCH_2$), 65.04 (Gal'-C6), 68.17 (OCH_2), 70.14, 70.63 (Gal-C4, Gal'-C4), 70.85 (Gal-C6), 72.43 (2C, Gal'-C2, Gal'-C3), 73.95 (Gal'-C5), 74.13 (Ph$\underline{C}H_2$), 74.63 (Gal-C5), 75.85 (Ph$\underline{C}H_2$), 80.00 (Gal-C2), 82.65 (Gal-C3), 104.20 (Gal-C1), 105.94 (Gal'-C1), 128.43, 128.50, 128.56, 129.03, 129.23, 129.32, 129.60, 129.74, 130.52, 130.72, 131.14, 131.46, 132.22, 134.30, 139.56, 140.10 (18C, 3×C_6H_5), 167.57 (CO);

HRMS (FAB) Calcd for $C_{38}H_{50}O_{12}Si$ $(M+NH_4)^+$: 744.3415; Found: m/z 744.3402.

Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**55**), (GG-031, *I-031*).

A suspension of **7** (300 mg, 0.563 mmol) and activated molecular sieves 3Å (200 mg) in DCM (3 mL) was stirred at rt for 3 h. Then TMSSMe (112 μL, 0.788 mmol) and TMSOTf (76.0 μL, 0.400 mmol) were added. The reaction was stirred at 45°C under argon for 5.5 h and at rt for further 16 h. The mixture was then diluted with DCM (5

mL) and filtered through a pad of Celite. The Celite was washed with DCM (3×5 mL), and the combined filtrates were washed with 5% $NaHCO_3$ (2×10 mL) and H_2O (10 mL). The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/AcOEt 1:4) to afford **55** (270 mg, 92%) as a mixture of α and β anomers with a ratio of 1:1 according to the ^{1}H NMR which was in accordance with Lit.[166]

Methyl (methyl 5-amino-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**65**), (GG-106, *I-106*).

To a stirred solution of **55** (100 mg, 0.192 mmol) in MeOH (8 mL) was added MsOH (124 μL, 1.92 mmol). The mixture was stirred for 24 h at 60°C and then neutralized with Et_3N. The solution was concentrated under reduced pressure, and the residue was purified by silica gel chromatography (DCM/MeOH 5:1 to 1:1) to afford **65** (60.0 mg, 100%) as colorless oil, with a ratio of α:β=1:1 according to the ^{1}H NMR which was in accordance to Lit.[167]

Methyl (methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**61**), (GG-107, *I-107*).

To a solution of **65** (60.0 mg, 0.192 mmol) and Et_3N (53.0 μL, 0.384 mmol) in MeOH (4 mL) was added CF_3COOMe (192 μL, 1.92 mmol). The mixture was stirred at rt for 2 h, then concentrated under reduced pressure. The resulting residue was stirred with Ac_2O (1.0 mL) and py (2.0 mL) at rt for 16 h, quenched with MeOH (2 mL), concentrated and co-evaporated with toluene (3×5 mL). The residue was purified by silica gel chromatography (PE/AcOEt 6:1) to afford **61** (69.0 mg, 55%) as white foam, the NMR data of which were in accordance with Lit.[161]

Methyl 2,4,7,8,9-penta-*O*-acetyl-5-(*N*-acetylacetamido)-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosynate (**66**), (GG-027, *I-027*).

A solution of **6** (1.00 g, 3.10 mmol) and $TsOH·H_2O$ (47.8 mg, 0.250 mmol) in IPA (25.0 mL, 0.229 mmol) was stirred at 65°C for 16 h. The reaction was then quenched by adding Et_3N (5 mL), concentrated and co-evaporated with toluene (3×10 mL). The residue was purified by silica gel chromatography (PE/AcOEt 2:3) to afford **66** (1.38

g, 80%) as a mixture of α and β anomers with a ratio of 1:8 according to the ^{1}H NMR which was in accordance with with Lit.[160]

Methyl [methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamido)-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid]onate (**62**), (GG-028, *I-028*).

A suspension of **66** (144 mg, 0.250 mmol) and activated molecular sieves 3Å (100 mg) in DCM (1.5 mL) was stirred at rt for 3 h, then TMSSMe (50.0 μL, 0.350 mmol) and TMSOTf (34.0 μL, 0.190 mmol) were added. The reaction was stirred at 45°C under argon for 6 h, and at rt for further 16 h. The mixture was diluted with DCM (5 mL), and filtered through a pad of Celite. The Celite was washed with DCM (3×5 mL), and the combined filtrates were washed with 5% $NaHCO_3$ (2×10 mL) and H_2O (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/AcOEt 1:1) to afford **62** (91.0 mg, 65%) as a mixture of α and β anomers with a ratio of 1:4, the NMR data of which were in accordance with Lit.[160]

5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid (**67**), (GG-104, *I-104*).

Neu5Ac **5** (5.00 g, 16.1 mmol) was stirred with Ac_2O (12.5 mL), py (25.0 mL) and *cat*. DMAP at rt for 16 h under argon. The solution was concentrated under reduced pressure and co-evaporated with toluene (3×15 mL) to afford **67** (9.00 g, 100%) as white foam, which was used in the next step without further purification.

Benzyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-galacto-2-nonulopyranosynate (**68**), (GG-105, *I-105*).

To a solution of **67** (9.00 g, 16.1 mmol) in DMF (85 mL) were added KF (2.30 g, 39.6 mmol) and BnBr (2.70 mL, 23.4 mmol). The mixture was stirred at rt for 20 h and then evaporated under reduced pressure. The resulting syrup was diluted with DCM (100 mL), filtered, washed with H_2O (3×50 mL) and dried over Na_2SO_4. After filtration and concentration, the residue was purified by silica gel chromatography (PE/AcOEt/MeOH 6:3:0.5) to afford **68** (8.70 g, 89%) as white foam, which was used in the next step without further purification.

Methyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**63**), (GG-111, *I-111*).

A suspension of **68** (75.0 mg, 123 μmol) and activated molecular sieves 3Å (200 mg) in DCM (2 mL) was stirred at rt for 2 h. Then TMSSMe (24.0 μL, 172 μmol) and TMSOTf (16.7 μL, 92.3 μmol) were added. The reaction was stirred at 50°C under argon for 5 h and at rt for further 16 h. The mixture was diluted with DCM (5 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3×5 mL) and the combined filtrate was washed with 5% $NaHCO_3$ (2×10 mL) and H_2O (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/AcOEt 1:1 to 1:1.25) to afford **63** (60.0 mg, 82%) as white foam.

Benzyl (5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosyl chloride)onate (**69**).

In a sealed vessel (Bombenrohr), AcCl (7.60 mL, 0.107 mol) was added at –20°C to a solution of **68** (3.84 g, 6.32 mmol) in DCM (25 mL), followed by *conc*. HCl (0.958 mL, 9.74 mmol). After stirring at rt for 16 h, the mixture was concentrated and the residue was diluted with DCM (50 mL) and washed with H_2O (3×50 mL), 5% $KHCO_3$ (3×50 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford **69** (3.50 g, 95%) as white foam, the ^{1}H NMR data of which were in accordance with Lit.[169]

***O*-Ethyl *S*-(benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)dithiocarbonate** (**64**).

A solution of **69** (500 mg, 0.853 mmol), potassium ethyl xanthogenate (164 mg, 1.02 mmol) and TBAHS (290 mg, 0.853 mmol) in AcOEt (5 mL) and 5% aq. $NaHCO_3$ (5 mL) was stirred at rt for 3 h. The mixture was diluted with AcOEt (30 mL), washed with H_2O (2×30 mL) and brine (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/AcOEt/EtOH 6:4:1) to afford **64** (514 mg, 90%) as white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21-1.24 (m, 3H, $CH_2C\underline{H}_3$), 1.89, 2.02, 2.12, 2.13 (4s, 15H, 5×CH_3-OAc), 2.03 (m, 1H, Sia-H3a), 2.67 (dd, $J_{3a,3b}$ = 12.9, $J_{3b,4}$ = 4.6 Hz, 1H,

Sia-H3b), 4.04 (q, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.4$ Hz, 1H, Sia-H5), 4.21 (dd, $J_{8,9a} = 6.0$, $J_{9a,9b} = 12.4$ Hz, 1H, Sia-H9a), 4.33 (dd, $J_{8,9b} = 2.6$, $J_{9a,9b} = 12.4$ Hz, 1H, Sia-H9b), 4.36-4.46 (m, 2H, C<u>H</u>$_2$-CH$_3$), 4.56 (dd, $J_{5,6} = 10.8$, $J_{6,7} = 2.0$ Hz, 1H, Sia-H6), 4.85 (m, 1H, Sia-H4), 5.20-5.24 (m, 3H, Sia-NH, C<u>H</u>$_2$Ph), 5.27 (td, $J_{7,8} = J_{8,9a} = 6.0$, $J_{8,9b} = 2.6$ Hz, 1H, Sia-H8), 5.32 (dd, $J_{6,7} = 2.0$, $J_{7,8} = 6.2$ Hz, 1H, Sia-H7), 7.33-7.39 (m, 5H, C$_6$H$_5$).

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-*O*-dibenzyl-β-D-galactopyranoside (**70**), (GG-102, *I-176*).

To a solution of **60** (170 mg, 0.234 mmol) and **55** (244 mg, 0.470 mmol) in CH_3CN (6 mL) was added activated powdered molecular sieves 3Å (2.00 g). The mixture was stirred at rt under argon for 6 h, then cooled to –30°C. To the stirred mixture were added NIS (211 mg, 0.940 mmol) and TfOH (8.2 μL, 94.0 μmol), and the 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 filtrate was successively washed with 20% aq. $Na_2S_2O_3$ (30 mL), 5% $KHCO_3$ (2×20 mL) and H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH 60:1) to afford **70** (150 mg, 54%) as white foam.

$[\alpha]_D$ +1.1°, (*c* = 0.80, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 1.00-1.03 (m, 2H, $SiCH_2$), 1.89, 2.00, 2.04, 2.06, 2.10 (5s, 15H, CH_3-NHAc, 4×CH_3-OAc), 2.10 (m, 1H, Sia-H3a), 2.66-2.69 (m, 2H, Sia-H3b, OH), 2.87 (bs, 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, $J_{2,3} = 11.6$, $J_{3,4} = 3.3$ 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, $J_{8,9a} = 5.6$, $J_{9a,9b} = 12.5$ Hz, 1H, Sia-H9a), 4.00-4.05 (m, 4H, Gal-H4, Gal'-H4, Sia-H5, OCH_{2b}), 4.10 (dd, $J_{5,6} = 10.8$, $J_{6,7} = 1.9$ Hz, 1H, Sia-H6), 4.11 (dd, $J_{2,3} = 9.5$, $J_{3,4} = 3.5$ Hz, 1H, Gal'-H3), 4.23 (dd, $J_{8,9b} = 2.6$, $J_{9a,9b} = 12.5$ Hz, 1H, Sia-H9b), 4.34 (d, $J_{1,2} = 7.6$ Hz, 1H, Gal-H1), 4.46 and 4.51 (A, B of AB, $J = 12.0$ Hz, 2H, PhC<u>H</u>$_2$), 4.53 (m, 1H, Gal'-H6a), 4.61 (dd, $J_{5,6b} = 4.8$, $J_{6a,6b} = 11.6$ Hz, 1H, Gal'-H6b), 4.63 (d, $J_{1,2} = 7.7$ Hz, 1H, Gal'-H1), 4.83 (m, 2H, PhC<u>H</u>$_2$), 4.97 (ddd, $J_{3b,4} = 4.6$, $J_{3a,4} = 12.0$, $J_{4,5} = 10.3$ Hz, 1H, Sia-H4), 5.23 (d, $J_{5,NH} = 9.7$ Hz, 1H, Sia-NH), 5.31 (dd, $J_{6,7} = 1.8$, $J_{7,8} = 8.6$ Hz, 1H, Sia-H7), 5.36 (ddd, $J_{7,8} = 8.4$, $J_{8,9a} =$

5.5, $J_{8,9b}$ = 2.6 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 -1.45 (3C, $SiMe_3$), 18.45 ($SiCH_2$), 20.69, 20.73, 20.82, 21.16, 23.18 (CH_3-NHAc, 4×CH_3-OAc), 37.31 (Sia-C3), 49.58 (Sia-C5), 53.18 (OCH_3), 62.29 (Sia-C9), 63.38 (Gal'-C6), 66.97 (Sia-C7), 67.18 (OCH_2), 67.80 (Gal-C3), 68.21 (Sia-C8), 68.57 (Sia-C4), 68.76, 69.38 (Gal-C4, Gal'-C4), 69.96 (Gal-C6), 72.16 (Sia-C6), 72.68 (Gal'-C5), 73.31 (Gal-C5), 73.51 (Ph$\underline{C}H_2$), 74.94 (Ph$\underline{C}H_2$), 76.11 (Gal-C3), 97.78 (Sia-C2), 102.85 (Gal-C1), 103.80 (Gal'-C1), 127.60, 128.22, 128.23, 128.37, 128.46, 129.65, 129.70, 138.24, 138.76 (18C, 3×C_6H_5), 168.34, 170.22 (7C, 7×CO);

ESI-MS: Calcd for $C_{58}H_{77}NO_{24}Si$ $(M+Na)^+$: 1222.45; Found: m/z 1222.31.

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)-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-*O*-dibenzyl-β-D-galactopyranoside (**72**), (GG-126, *I-126*).

To a solution of **60** (82.8 mg, 114 μmol) and **63** (136 mg, 228 μmol) in CH_3CN (3 mL) was added activated powdered molecular sieves 3Å (1.00 g). The mixture was stirred at rt under argon for 4 h, then cooled to –30°C. To the stirred mixture were subsequently added NIS (102 mg, 456 μmol) and TfOH (4 μL, 45.6 μmol), and 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 (3×5 mL), the combined filtrate was successively washed with 20% aq. $Na_2S_2O_3$ (20 mL), 5% $KHCO_3$ (2×20 mL) and H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH 50:1) to afford **72** (74.6 mg, 51%) as white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 1.00-1.03 (m, 2H, $SiCH_2$), 1.90, 1.99, 2.04, 2.05, 2.12 (5s, 15H, CH_3-NHAc, 4×CH_3-OAc), 2.07 (m, 1H, Sia-H3a), 2.41 (bs, 1H, Gal'-4OH), 2.74 (dd, $J_{3a,3b}$ = 13.1, $J_{3b,4}$ = 4.6 Hz, Sia-H3b), 3.40 (bs, 1H, Gal-4OH), 3.50-3.53 (m, 2H, Gal-H5, Gal-H6a), 2.57 (m, 1H, OCH_2-Ha), 3.64-3.75 (m, 5H, Gal-H2, Gal-H3, Gal-H6b, Gal'-H2, Gal'-H5), 3.98-4.03 (m, 4H, Gal-H4, Sia-H5, Sia-H9a, OCH_2-Hb), 4.05 (dd, $J_{2,3}$ = 7.0, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H3), 4.10 (dd, $J_{5,6}$ = 10.8, $J_{6,7}$ = 1.8 Hz, 1H, Sia-H6), 4.23 (dd, $J_{8,9b}$ = 2.6, $J_{9a,9b}$ = 12.5 Hz, 1H, Sia-H9b),

4.33 (d, $J_{1,2}$ = 7.6 Hz, 1H, Gal-H1), 4.37-4.42 (m, 2H, Gal'-H6a, Gal'-H6b), 4.44, 4.49 (A, B of AB, J = 12.0 Hz, 2H, PhC$\underline{H}_2$), 4.60 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal'-H1), 4.81, 4.85 (A, B of AB, J = 11.0 Hz, 2H, PhC$\underline{H}_2$), 4.96 (ddd, $J_{3b,4}$ = 4.6, $J_{3a,4}$ = 11.9, $J_{4,5}$ = 10.2 Hz, 1H, Sia-H4), 5.16-5.21 (m, 2H, PhC$\underline{H}_2$-HA, Sia-NH), 5.24 (B of AB, J = 11.8 Hz, 1H, PhC$\underline{H}_2$-HB), 5.31 (dd, $J_{6,7}$ = 1.8, $J_{7,8}$ = 8.6 Hz, 1H, Sia-H7), 5.36 (m, 1H, Sia-H8), 7.21-7.33, 7.37-7.52, 7.52-7.53, 8.01-8.03 (m, 20H, 4×C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.45 (3C, $SiMe_3$), 18.41 ($SiCH_2$), 20.67, 20.73, 20.80, 21.21 (5C, CH_3-NHAc, 4× CH_3-OAc), 37.53 (Sia-C3), 49.49 (Sia-C5), 62.24 (Sia-C9), 63.63 (Gal'-C6), 66.92 (Sia-C7), 67.13 (OCH_2), 67.73 (Gal'-C4), 68.26 (Sia-C8), 68.35 (Ph$\underline{C}H_2$), 68.72 (Sia-C4), 69.33 (Gal-C4), 70.06 (Gal-C6), 72.09 (Gal'-C2), 72.74 (2C, Gal'-C5, Sia-C6), 73.35 (Gal-C5), 73.47 (Ph$\underline{C}H_2$), 74.94 (Ph$\underline{C}H_2$), 76.22 (Gal'-C3), 78.00 (Gal-C2), 83.11 (Gal-C3), 97.67 (Sia-C2), 102.77 (Gal-C1), 103.61 (Gal'-C1), 127.49, 127.55, 128.16, 128.21, 128.34, 128.34, 128.44, 128.84, 128.94, 129.24, 129.65, 129.73, 130.89, 133.23, 134.14, 138.27, 138.82 (24C, 4×C_6H_5), 166.17, 167.59, 169.95, 170.11, 170.30, 170.56, 170.74 (7×CO).

2-(Trimethylsilyl)ethyl (benzyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-6-*O*-benzoyl-β-D-galactopyranoside (**74**), (GG-088, *I-088*).

To a stirred solution of **64** (50.0 mg, 74.4 μmol) and **3** (43.0 mg, 112 μmol) in CH_3CN/DCM (3:2, 2.5 mL) was added activated powdered molecular sieves 3 Å (200 mg). The reaction mixture was stirred at rt under argon for 5 h and then cooled to –70°C. After the addition of NIS (31.8 mg, 0.141 mmol) and TfOH (1.24 μL, 14.1 μmol), stirring was continued for 16 h at –70°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), the combined filtrates were successively washed with 20% $Na_2S_2O_3$ (20 mL), saturated $KHCO_3$ (2×20 mL) and H_2O (20 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (DCM/MeOH 60:1) to afford **74** (17.8 mg, 26%) as white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ -0.02 (s, 9H, $SiMe_3$), 0.99-1.13(m, 2H, $SiCH_2$), 1.89, 2.03, 2.04, 2.10, 2.14 (5s, 15H, CH_3-NHAc, 4×CH_3-OAc), 2.02 (m, 1H, Sia-H3a), 2.69 (bs, 1H, Gal-2-OH), 2.76 (dd, $J_{3a,3b}$ = 12.9, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.31 (m, 1H, Gal-H5), 3.63-3.69 (m, 3H, Gal-H2, Gal-H6a, OCH_2-Ha), 3.97-4.08 (m, 4H, Gal-

H6b, Sia-H5, Sia-H9a, OCH_2-Hb), 4.10 (dd, $J_{5,6}$ = 10.8, $J_{6,7}$ = 1.9 Hz, 1H, Sia-H6), 4.25 (dd, $J_{8,9b}$ = 2.7, $J_{9a,9b}$ = 12.5 Hz, 1H, Sia-H9b), 4.39-4.42 (m, 3H, Gal-H1, Gal-H3, Gal-H4), 4.93 (ddd, $J_{3a,4}$ = 12.1, $J_{3b,4}$ = 4.6, $J_{4,5}$ = 10.2 Hz, 1H, Sia-H4), 5.14-5.17 (m, 2H, PhC$\underline{H}_2$-HA, Sia-NH), 5.20 (B of AB, J = 11.9 Hz, 1H, PhC$\underline{H}_2$-HB), 5.32 (dd, $J_{6,7}$ = 1.9, $J_{7,8}$ = 9.0 Hz, 1H, Sia-H7), 5.45 (ddd, $J_{7,8}$ = 9.0, $J_{8,9a}$ = 5.1, $J_{8,9b}$ = 2.7 Hz, 1H, Sia-H8), 7.35-7.43, 7.54-7.57, 8.02-8.04 (m, 10H, 2×C_6H_5).

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-*O*-dibenzyl-β-D-galactopyranoside (**77**), (GG-168, *I-144*).

To a stirred solution of **70** (100 mg, 83.1 μmol) in py (1.0 mL) at 0°C was added Ac_2O (0.5 mL). The mixture was stirred for 16 h at rt under argon, and then quenched by MeOH (1 mL). The solution was concentrated under reduced pressure and co-evaporated with toluene (3×10 mL). The residue was purified by silica gel chromatography (toluene/AcOEt 1:1) to afford **77** (87.6 mg, 80%) as white foam.

$[\alpha]_D$ -13.2°, (*c*=0.63, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.99-1.07 (m, 2H, $SiCH_2$), 1.68 (t, $J_{3a,3b}$ = $J_{3a,4}$ = 12.5 Hz, 1H, Sia-H3a), 1.83, 1.90, 1.98, 1.99, 2.00, 2.10, 2.11, 2.12 (8s, 24H, CH_3-NHAc, 7×CH_3-OAc), 2.58 (dd, $J_{3a,3b}$ = 12.7, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.47-3.58 (m, 3H, Gal-H6a, Gal-H6b, OCH_2-Ha), 3.61 (dd, $J_{5,6}$ = 10.9, $J_{6,7}$ = 2.6 Hz, 1H, Sia-H6), 3.64 (dd, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.6 Hz, 1H, Gal-H2), 3.72 (m, 1H, Gal-H5), 3.73 (s, 3H, OCH_3), 3.88 (dd, $J_{2,3}$ = 9.6, $J_{3,4}$ = 3.6 Hz, 1H, Gal-H3), 3.94 (dd, $J_{8,9a}$ = 5.9, $J_{9a,9b}$ = 12.5 Hz, 1H, Sia-H9a), 4.02-4.04 (m, 3H, Gal'-H5, Sia-H5, OCH_2-Hb), 4.18 (dd, $J_{5,6a}$ = 7.8, $J_{6a,6b}$ = 11.0 Hz, 1H, Gal'-H6a), 4.30 (dd, $J_{8,9b}$ = 2.6, $J_{9a,9b}$ = 12.5 Hz, 1H, Sia-H9b), 4.41 (m, 1H, Gal'-H6b), 4.42 (d, $J_{1,2}$ = 8.0 Hz, 1H, Gal-H1), 4.48 and 4.51 (A, B of AB, J = 11.8 Hz, 2H, PhC$\underline{H}_2$), 4.59 (dd, $J_{2,3}$ = 10.2, $J_{3,4}$ = 3.2 Hz, 1H, Gal'-H3), 4.75 and 4.83 (A, B of AB, J = 11.0 Hz, 2H, PhC$\underline{H}_2$), 4.84 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal'-H1), 4.88 (m, 1H, Sia-H4), 5.05 (d, $J_{3,4}$ = 3.2 Hz, 1H, Gal'-H4), 5.11 (dd, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.1 Hz, 1H, Gal'-H2), 5.21 (d, $J_{5,NH}$ = 10.2 Hz, 1H, Sia-NH), 5.34 (dd, $J_{6,7}$ = 2.6, $J_{7,8}$ = 8.9 Hz, 1H, Sia-H7), 5.46 (d, $J_{3,4}$ = 3.6 Hz, 1H, Gal-H4), 5.50 (ddd, $J_{7,8}$ = 8.8, $J_{8,9a}$ = 5.9, $J_{8,9b}$ = 2.7 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 -1.57 (3C, $SiMe_3$), 18.39 ($SiCH_2$), 20.62, 20.66, 20.69, 20.70, 20.87 (8C, CH_3-NHAc, 7× CH_3-OAc), 37.43 (Sia-C3), 49.01 (Sia-C5), 52.94 (OCH_3), 67.06, 67.22 (Gal'-C4, Sia-C7), 67.71, 67.76 (Sia-C8, OCH_2), 69.22, 69.25, 69.43 (Gal-C4, Gal-C6, Sia-C4), 70.13, 70.39 (Gal'-C2, Gal'-C5), 71.45, 72.02, 72.92 (Gal-C5, Gal'-C3, Sia-C6), 73.59 (Ph$\underline{C}H_2$), 75.09 (Ph$\underline{C}H_2$), 78.57 (Gal-C2), 80.16 (Gal-C3), 96.64 (Sia-C2), 100.71 (Gal'-C1), 103.01 (Gal-C1), 127.35, 127.59, 127.66, 127.70, 128.09, 128.14, 128.21, 128.30, 128.38, 128.75, 128.95, 129.61, 129.77, 130.84, 133.01, 137.77, 137.93, 138.85 (18C, 3×C_6H_5), 165.57, 167.89, 169.55, 169.62, 170.15, 170.21, 170.27, 170.45, 170.52, 170.79 (10×CO);
ESI-MS: Calcd for $C_{64}H_{83}NNaO_{27}Si$ $(M+Na)^+$: 1348.48; Found: m/z 1348.27.

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 (**7 6**) and **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)-β-D-galactopyranoside** (**79**), (GG-183-2 and GG-183-4, *I-183*).

77 (81.0 mg, 61.1 μmol) in EtOH (4.0 mL) and AcOH (0.8 mL) was hydrogenolyzed (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. The combined filtrates were concentrated and the residue was purified by silica gel chromatography (DCM/MeOH 50:1) to afford **76** (53.5mg, 76%) and **79** (10.0 mg, 15%) as white foams.

76: $[\alpha]_D$ -2.8°, (*c* = 0.68, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.91-1.21 (m, 2H, $SiCH_2$), 1.71 (t, $J_{3a,3b} = J_{3a,4} = 12.4$ Hz, 1H, Sia-H3a), 1.84, 1.99, 2.04, 2.07, 2.13, 2.21 (7s, 24H, CH_3-NHAc, 7×CH_3-OAc), 2.57 (dd, $J_{3a,3b} = 12.6$, $J_{3b,4} = 4.6$ Hz, 1H, Sia-H3b), 2.94 (bs, 1H, OH), 3.44 (m, 1H, Gal-H6a), 3.55-3.60 (m, 3H, Gal-H5, Gal-H6b, OCH_2-Ha), 3.62 (dd, $J_{5,6} = 10.6$, $J_{6,7} = 2.7$ Hz, 1H, Sia-H6), 3.73 (s, 3H, OCH_3), 3.78-3.79 (m, 2H, Gal-H2, Gal-H3), 3.93 (dd, $J_{8,9a} = 6.7$, $J_{9a,9b} = 12.2$ Hz, 1H, Sia-H9a), 3.97-4.00 (m, 2H, Gal'-H5, OCH_2-Hb), 4.06 (q, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.4$ Hz, 1H, Sia-H5), 4.16 (dd, $J_{5,6a} = 7.2$, $J_{6a,6b} = 11.2$ Hz, 1H, Gal'-H6a), 4.32 (d, $J_{1,2} = 7.6$ Hz, 1H, Gal-H1), 4.32-4.36 (m, 2H, Gal'-H6b, Sia-H9b), 4.58 (dd, $J_{2,3} =$

10.2, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H3), 4.85 (ddd, $J_{3b,4}$ = 4.6, $J_{3a,4}$ = $J_{4,5}$ = 12.0 Hz, 1H, Sia-H4), 4.91 (d, $J_{1,2}$ = 7.9 Hz, 1H, Gal'-H1), 5.01-5.04 (m, 2H, Gal'-H2, Gal'-H4), 5.20 (d, $J_{5,NH}$ = 10.3 Hz, 1H, Sia-NH), 5.28 (d, $J_{3,4}$ = 2.4 Hz, 1H, Gal-H4), 5.32 (dd, $J_{6,7}$ = 2.7, $J_{7,8}$ = 9.3 Hz, 1H, Sia-H7), 5.62 (ddd, $J_{7,8}$ = 9.3, $J_{8,9a}$ = 6.7, $J_{8,9b}$ = 2.7 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 -1.51 (3C, $SiMe_3$), 18.21 ($SiCH_2$), 20.66, 20.72, 20.76, 20.80, 20.94 (8C, CH_3-NHAc, 7×CH_3-OAc), 37.37 (Sia-C3), 48.93 (Sia-C5), 52.97 (OCH_3), 59.98 (Gal-C6), 61.51 (Gal'-C6), 62.91 (Sia-C9), 67.28, 67.33, 67.39 (Gal'-C4, Sia-C7, Sia-C8), 67.65 (OCH_2), 69.24, 69.32 (Gal-C4, Sia-C4), 69.84 (Gal'-C2), 70.59, 70.74, 70.96 (Gal-C2, Gal'-C3, Gal'-C5), 71.87 (Sia-C6), 73.24 (Gal-C5), 79.70 (Gal-C3), 96.66 (Sia-C2), 101.15 (Gal'-C1), 102.52 (Gal-C1), 128.15, 128.28, 129.64, 129.72, 133.11 (C_6H_5-<u>C</u>H), 165.68, 165.82, 167.88, 169.77, 170.12, 170.33, 170.62, 170.88, 170.96, 172.67 (11C, C_6H_5-<u>C</u>, 10×CO);

HRMS (FAB) Calcd for $C_{50}H_{71}NO_{27}Si$ $(M+H)^+$: 1146.4061; Found: m/z 1146.4102;

79: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.93-1.06 (m, 2H, $SiCH_2$), 1.73 (t, $J_{3a,3b}$ = $J_{3a,4}$ = 12.5 Hz, 1H, Sia-H3a), 1.85 (s, 3H, CH_3-NHAc), 2.01, 2.04, 2.08, 2.13, 2.15, 2.21 (6s, 18H, 6×CH_3-OAc), 2.59 (dd, $J_{3a,3b}$ = 12.6, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 2.74 (bs, 1H, OH), 2.84 (bs, 1H, OH), 3.45 (t, $J_{5,6a}$ = $J_{5,6b}$ = 5.7 Hz, 1H, Gal-H5), 3.47-3.65 (m, 4H, Gal-H3, Gal-H6a, Sia-H6, OCH_2-Ha), 3.75 (m, 1H, Gal-H2), 3.80 (s, 3H, OCH_3), 3.86 (dd, $J_{5,6b}$ = 6.7, $J_{6a,6b}$ = 11.8 Hz, 1H, Gal-H6), 3.96 (dd, $J_{8,9a}$ = 6.2, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9a), 3.98 (d, $J_{3,4}$ = 4.2 Hz, 1H, Gal-H4), 4.00-4.09 (m, 3H, Gal'-H5, Sia-H5, OCH_2-Hb), 4.26 (d, $J_{1,2}$ = 7.9 Hz, 1H, Gal-H1), 4.28 (m, 1H, Gal'-H6a), 4.34 (dd, $J_{8,9b}$ = 2.8, $J_{9a,9b}$ = 12.3 Hz, Sia-H9b), 4.37 (dd, $J_{5,6b}$ = 7.6, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal'-H6b), 4.62 (dd, $J_{2,3}$ = 10.2, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H3), 4.88 (m, 1H, Sia-H4), 4.90 (d, $J_{1,2}$ = 8.0 Hz, 1H, Gal'-H1), 5.02 (d, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H4), 5.08 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.1 Hz, 1H, Gal'-H2), 5.17 (d, $J_{5,NH}$ = 10.2 Hz, 1H, Sia-NH), 5.34 (dd, $J_{6,7}$ = 2.6, $J_{7,8}$ = 9.4 Hz, 1H, Sia-H7), 5.61 (ddd, $J_{7,8}$ = 9.2, $J_{8,9a}$ = 6.1, $J_{8,9b}$ = 2.9 Hz, 1H, Sia-H8), 7.44-7.47, 7.57-7.60, 8.02-8.03 (m, 5H, C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.45 (3C, $SiMe_3$), 18.21 ($SiCH_2$), 20.69, 20.76, 20.79, 20.81 (7C, CH_3-NHAc, 6×CH_3-OAc), 37.42 (Sia-C3), 49.00 (Sia-C5), 53.13 (OCH_3), 62.12 (Gal'-C6), 62.43 (Gal-C6), 62.74 (Sia-C9), 67.18 (OCH_2), 67.37, 67.59 (Sia-C7, Sia-C8), 68.13, 68.44 (Gal-C4, Gal'-C4), 69.23 (Sia-C4), 69.73 (Gal'-C2), 70.18 (Gal-C2), 70.83 (Gal'-C3), 71.05 (Gal'-C5), 71.90 (Sia-C6), 73.85 (Gal-C5),

83.05 (Gal-C3), 96.70 (Sia-C2), 101.60 (Gal'-C1), 102.39 (Gal-C1), 128.45, 129.67, 133.41 (6C, C_6H_5), 165.84, 167.97, 169.79, 170.38, 170.40, 170.75, 170.92 (9C, 9×CO);

ESI-MS: Calcd for $C_{48}H_{69}NNaO_{26}Si$ $(M+Na)^+$: 1126.38; Found: m/z 1126.56.

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** (**80α** and **80β**), (GG-186-5 and GG-186-2, *I-186*).

To a solution of **76** (61.3 mg, 53.5 μmol) and **55** (70.0 mg, 13.4 μmol) in CH_3CN (5 mL) was added activated powdered molecular sieves 3Å (2.00 g). The mixture was stirred at rt under argon for 6 h and then cooled to –40°C. To the stirred mixture were subsequently added NIS (60.0 mg, 0.267 mmol) and TfOH (2.4 μL, 26.7 μmol), and 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), the combined filtrates were successively washed with 20% aq. $Na_2S_2O_3$ (20 mL), 5% aq. $KHCO_3$ (2×10 mL) and H_2O (10 mL), dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH 35:1) to afford **80α** (33.0 mg, 38%) and **80β** (10.0 mg, 12%) as white foams.

80α: $[\alpha]_D$ –14.0°, (*c* = 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.98-1.15 (m, 2H, $SiCH_2$), 1.69 (t, $J_{3a,3b} = J_{3a,4} = 12.3$ Hz, 1H, Sia-H3a), 1.83, 1.85 (2s, 6H, 2×CH_3-NHAc), 1.90 (t, $J_{3a,3b} = J_{3a,4} = 12.6$ 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×OAc), 2.55 (m, 2H, Sia-H3b, Sia'-H3b), 3.18 (bs, 1H, OH), 3.28 (dd, $J_{5,6a} = 6.3$, $J_{6a,6b} = 9.6$ Hz, 1H, Gal-H6a), 3.46-3.67 (m, 3H, Sia-H6, Sia'-H6, OCH_2-Ha), 3.68-3.78 (m, 2H, Gal-H2, Gal-H5), 3.72 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.79-3.84 (m, 2H, Gal-H3, Gal-H6b), 3.91 (dd, $J_{8,9a} = 7.2$, $J_{9a,9b} = 12.2$ Hz, 1H, Sia-H9a), 3.96 (t, $J_{5,6a} = J_{5,6b} = 7.0$ Hz, 1H, Gal'-H5), 4.00-4.04 (m, 3H, Sia-H5, Sia'-H5, OCH_2-Hb), 4.07 (dd, $J_{8,9a} = 5.3$, $J_{9a,9b} = 11.9$ Hz, 1H, Sia'-H9a), 4.18 (m, 1H, Gal'-H6a), 4.27 (m, 1H, Sia'-H9b), 4.28 (d, $J_{1,2} = 7.6$ Hz, 1H, Gal-H1), 4.34-4.40 (m, 2H, Gal'-H6b, Sia-H9b), 4.55 (dd, $J_{2,3} = 10.0$, $J_{3,4} = 3.4$ 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, $J_{1,2}$ = 8.0 Hz, 1H, Gal'-H1), 5.12 (d, $J_{5,NH}$ = 10.2 Hz, 1H, Sia-NH), 5.18 (d, $J_{5,NH}$ = 8.0 Hz, 1H, Sia'-NH), 5.27-5.34 (m, 3H, Sia-H7, Sia'-H7, Sia'-H8), 5.39 (d, $J_{3,4}$ = 3.5 Hz, 1H, Gal-H4), 5.61 (ddd, $J_{7,8}$ = 9.5, $J_{8,9a}$ = 7.3, $J_{8,9b}$ = 2.8 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 -1.46 (3C, $SiMe_3$), 18.22 ($SiCH_2$), 20.69, 20.73, 20.79, 20.88, 20.94 (13C, 2×CH_3-NHAc, 11×CH_3-OAc), 37.70, 38.67 (Sia-C3, Sia'-C3), 48.97, 49.38 (Sia-C5, Sia'-C5), 52.82, 52.97 (2×OCH_3), 61.37 (Gal'-C6), 62.27 (Sia'-C9), 62.94, 63.07 (Gal-C6, Sia-C9), 67.26, 67.35 (3C, Gal'-C4, Sia-C8, Sia-C7), 67.52 (OCH_2), 68.94, 69.26, 69.29, 70.57 (Sia-C4, Sia'-C4, Sia'-C7, Sia'-C8), 71.25, 71.34, 71.96, 72.05, 72.58 (7C, Gal-C2, Gal-C5, Gal'-C2, Gal'-C3, Gal'-C5, Sia-C6, Sia'-C6), 77.80 (Gal-C3), 96.70, 98.69 (Sia-C2, Sia'-C2), 100.10 (Gal'-C1), 102.40 (Gal-C1), 128.29, 129.70, 133.11 (6C, C_6H_5), 165.67, 167.72, 169.63, 169.71, 169.99, 170.13, 170.21, 170.34, 170.59, 170.94, 171.16 (16C, 16×CO);

HRMS (FAB) Calcd for $C_{70}H_{98}N_2O_{39}Si$ $(M+NH_4)^+$: 1636.5860; Found: m/z 1636.5869;

80β: $[\alpha]_D$ -12.7°, (*c* = 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.93-1.15 (m, 2H, $SiCH_2$), 1.67-1.77 (m, 2H, Sia-H3a, Sia'-H3a), 1.83 (s, 6H, 2×CH_3-NHAc), 1.92, 1.99, 2.01, 2.03, 2.04, 2.07, 2.08, 2.12, 2.13, 2.21, 2.22 (11s, 33H, 11×CH_3-OAc), 2.40 (dd, $J_{3a,3b}$ = 12.8, $J_{3b,4}$ = 5.0 Hz, 1H, Sia'-H3b), 2.57 (dd, $J_{3a,3b}$ = 12.6, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.07 (bs, 1H, OH), 3.30 (t, $J_{5,6a}$ = $J_{6a,6b}$ = 9.3 Hz, 1H, Gal-H6a), 3.40 (dd, $J_{5,6b}$ = 4.6, $J_{6a,6b}$ = 8.7 Hz, 1H, Gal-H6b), 3.57 (m, 1H, OCH_2-Ha), 3.62 (dd, $J_{5,6}$ = 10.7, $J_{6,7}$ = 2.7Hz, 1H, Sia-H6), 3.71 (dd, $J_{5,6}$ = 10.6, $J_{6,7}$ = 2.5 Hz, 1H, Sia'-H6), 3.73-3.83 (m, 3H, Gal-H2, Gal-H3, Gal-H5), 3.76 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.92 (dd, $J_{8,9a}$ = 7.2, $J_{9a,9b}$ = 12.2 Hz, 1H, Sia-H9a), 3.95-3.99 (m, 2H, Gal'-H5, OCH_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, $J_{1,2}$ = 7.6 Hz, 1H, Gal-H1), 4.37 (dd, $J_{8,9b}$ = 2.8, $J_{9a,9b}$ = 12.2 Hz, 1H, Sia-H9b), 4.45 (dd, $J_{5,6b}$ = 5.6, $J_{6a,6b}$ = 10.9 Hz, 1H, Gal'-H6b), 4.56 (dd, $J_{2,3}$ = 10.2, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H3), 4.73 (dd, $J_{8,9b}$ = 2.3, $J_{9a,9b}$ = 12.3 Hz, 1H, Sia'-H9b), 4.84 (m, 1H, Sia-H4), 4.98-5.00 (m, Gal'-H1, Gal'-H2, Sia'-H8), 5.05 (d, $J_{3,4}$ = 3.2 Hz, 1H, Gal'-H4), 5.10 (d, $J_{5,NH}$ = 10.3 Hz, 1H, Sia-NH), 5.30 (dd, $J_{6,7}$ = 2.7, $J_{7,8}$ = 9.1 Hz, 1H, Sia-H7), 5.33-5.35 (m, 2H, Sia'-H4, Sia'-H7), 5.59 (d, $J_{3,4}$ = 3.1 Hz, 1H, Gal-H4), 5.63 (ddd, $J_{7,8}$ = 9.5, $J_{8,9a}$ = 7.2, $J_{8,9b}$ = 2.8 Hz, 1H, Sia-H8), 6.22 (d, $J_{5,NH}$ = 10.2 Hz, 1H, Sia'-NH), 7.38-7.41, 7.51-7.54, 7.97-7.98 (m, 5H, C_6H_5);

^{13}C NMR (500MHz, $CDCl_3$): δ -1.33 (3C, $SiMe_3$), 18.41 ($SiCH_2$), 20.84, 20.87, 20.92, 20.97, 21.11, 21.38, 21.50 (13C, 2×CH_3-NHAc, 11×CH_3-OAc), 37.21, 37.44 (Sia-C3, Sia'-C3), 47.81 (Sia'-C5), 49.12 (Sia-C5), 53.01, 53.11 (2×OCH_3), 60.19 (Gal-C6), 61.26 (Gal'-C6), 62.61 (Sia'-C9), 63.21 (Sia-C9), 67.46 (Gal'-C4), 67.52 (Sia-C7), 67.66 (OCH_2), 68.24 (Sia'-C7), 68.35 (Sia-C8), 68.96, 68.99 (Gal-C4, Sia'-C4), 69.35 (Sia-C4), 70.06 (Sia'-C8), 70.56 (Gal'-C3), 71.24, 71.31 (Gal-C2, Gal'-C5), 72.13, 72.27, (3C, Gal-C5, Sia-C6, Sia'-C6), 72.52 (Gal'-C2), 79.12 (Gal-C3), 96.87, 98.13 (Sia-C2, Sia'-C2), 101.02 (Gal'-C1), 102.94 (Gal-C1), 128.88, 30.96, 133.17 (6C, C_6H_5), 165.70, 167.65, 168.04, 170.00, 170.18, 170.26, 170.35, 170.39, 170.48, 170.62, 170.70, 170.73, 170.80, 171.03, 171.36, 172.28 (16C, 16×CO).

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 (54α), (GG-162, *I-162*).

To a solution of **80α** (30.0 mg, 18.5 μmol) in MeOH (2.5 mL) was added freshly prepared 1M NaOMe/MeOH (0.3 mL). The mixture was stirred at rt under argon for 7 h, then H_2O (1 mL) was added and stirring was continued for 16 hr at rt. The solution was concentrated and the residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **54α** as white solid (9.6mg, 48.5%) after a final lyophilization from H_2O.

$[\alpha]_D$ +1.5°, (*c* = 0.64, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.00 (s, 9H, $SiMe_3$), 0.95 (td, *J* = 12.9, *J* = 5.2 Hz, 1H, $SiCH_2$-Ha), 1.04 (td, *J* = 12.9, *J* = 5.5 Hz, 1H, $SiCH_2$-Hb), 1.63 (t, $J_{3a,3b} = J_{3a,4} = 12.1$ Hz, 1H, Sia'-H3a), 1.77 (t, $J_{3a,3b} = J_{3a,4} = 12.1$ Hz, 1H, Sia-H3a), 2.00 (s, 6H, 2×CH_3-NHAc), 2.68 (dd, $J_{3a,3b} = 12.4$, $J_{3b,4} = 4.6$ Hz, 1H, Sia'-H3b), 2.73 (dd, $J_{3a,3b} = 12.4$, $J_{3b,4} = 4.6$ Hz, 1H, Sia-H3b), 3.58-3.68 (m, 13H, Gal-H2, Gal-H6a, Gal'-H2, Gal'-H6a, Gal'-H6b, 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, OCH_2-Ha), 3.89-3.92 (m, 2H, Gal-H6b, Gal'-H4), 4.00 (ddd, *J* = 10.1, *J* = 12.7, *J* = 5.2 Hz, 1H, OCH_2-Hb), 4.08 (dd, $J_{2,3} = 9.8$, $J_{3,4} = 3.1$ Hz, 1H, Gal'-H3), 4.18 (d, $J_{3,4} = 3.4$ Hz, 1H, Gal-H4), 4.42 (d, $J_{1,2} = 8.0$ Hz, 1H, Gal-H1), 4.63 (d, $J_{1,2} = 7.8$ Hz, 1H, Gal'-H1);

^{13}C NMR (500 MHz, D_2O): δ -2.56 (3C, $SiMe_3$), 17.51 ($SiCH_2$), 21.95, 21.97 (2×CH_3-NHAc), 39.62, 40.26 (Sia-C3, Sia'-C3), 51.60, 51.80 (Sia-C5, Sia'-C5), 60.86 (Gal'-C6), 62.47 (Sia'-C9), 62.53 (Sia-C9), 63.11 (Gal-C6), 67.36 (Gal'-C4), 68.02, 68.13, 68.27 (Sia-C4, Sia-C7, Sia'-C4), 68.33 (Gal-C4), 68.39 (O$\underline{C}H_2$), 69.40, 69.60 (3C, Sia'-C7, Gal'-C5, Gal-C5), 71.59, 71.73 (Sia-C6, Sia'-C6), 72.56, 72.78 (Sia-C8, Sia'-C8), 74.78 (2C, Gal-C2, Gal'-C2), 75.51 (Gal'-C3), 82.59 (Gal-C3), 99.71, 100.34 (Sia-C2, Sia'-C2), 101.67 (Gal-C1), 104.08 (Gal'-C1), 173.38, 173.80, 174.92, 174.99 (4×CO).

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*-β-D-*galacto*-2-nonulopyranosynate)]-(2→6)-β-D-galactopyranoside (**54β**), (GG-228, *I-228*).

To a solution of **80β** (10.0 mg, 6.20 μmol) in MeOH (1.5 mL) was added freshly prepared 1M NaOMe/MeOH (0.2 mL,). The mixture was stirred at rt under argon for 7 h, then H_2O (0.25 mL) was added and the mixture was stirred for 16 hr at rt. The solution was concentrated and the residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **54β** as white solid (4.50 mg, 68%) after a final lyophilization from H_2O.

$[\alpha]_D$ -2.9°, (*c* = 0.3, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.00 (s, 9H, $SiMe_3$), 0.95 (td, *J* = 13.0, *J* = 5.1 Hz, 1H, $SiCH_2$-Ha), 1.05 (td, *J* = 13.0, *J* = 5.4 Hz, 1H, $SiCH_2$-Hb), 1.61 (t, $J_{3a,3b} = J_{3a,4} = 12.2$ Hz, 1H, Sia'-H3a), 1.78 (t, $J_{3a,3b} = J_{3a,4} = 12.1$ Hz, 1H, Sia-H3a), 2.00, 2.02 (2s, 6H, 2×CH_3-NHAc), 2.33 (dd, $J_{3a,3b} = 12.9$, $J_{3b,4} = 4.6$ Hz, 1H, Sia'-H3b), 2.74 (dd, $J_{3a,3b} = 12.3$, $J_{3b,4} = 4.4$ Hz, 1H, Sia-H3b), 3.41 (dd, $J_{5,6a} = 6.2$, $J_{6a,6b} = 9.2$ Hz, 1H, Gal-H6a), 3.49 (d, $J_{5,6} = 9.4$ Hz, 1H, Sia'-H6), 3.56-3.70 (m, 9H, Gal-H2, Gal-H6b, Gal'-H2, Gal'-H5, Sia-H8, Sia-H9a, Sia'-H4, Sia'-H8, Sia'-H9a), 3.71-3.76 (m, 3H, Gal'-H6a, Gal'-H6b, OCH_2-Ha), 3.78-3.89 (m, 9H, Gal-H3, Gal-H5, Sia-H5, Sia-H6, Sia-H7, Sia-H9b, Sia'-H5, Sia'-H7, Sia'-H9b), 3.93 (d, $J_{3,4} = 2.7$ Hz, 1H, Gal'-H4), 3.97-4.04 (m, 2H, Sia-H4, OCH_2-Hb), 4.09 (dd, $J_{2,3} = 9.8$, $J_{3,4} = 2.9$ Hz, 1H, Gal'-H3), 4.22 (d, $J_{3,4} = 2.8$ Hz, 1H, Gal-H4), 4.46 (d, $J_{1,2} = 8.0$ Hz, 1H, Gal-H1), 4.67 (d, $J_{1,2} = 7.9$ Hz, 1H, Gal'-H1);

^{13}C NMR (500 MHz, D_2O): δ -2.60 (3C, $SiMe_3$), 17.51 ($SiCH_2$), 21.97, 22.07 (2×CH_3-NHAc), 39.61, 39.70 (Sia-C3, Sia'-C3), 51.60, 51.84 (Sia-C5, Sia'-C5), 60.79 (Gal'-C6), 61.03 (Gal-C6), 62.49, 63.41 (Sia-C9, Sia'-C9), 67.09 (Sia-C4), 67.40 (Gal'-C4), 68.02 (Sia'-C8), 68.32 (Sia-C8), 68.40 (O$\underline{C}H_2$), 68.43 (2C, Gal-C4, Sia'-C6), 69.49 (Sia'-C4), 69.72 (Sia-C6), 70.13 (Sia'-C7), 70.30 (Sia-C7), 71.76 (Gal-C5), 72.79, 72.83 (Gal-C2, Gal'-C2), 74.83 (Gal'-C5), 75.52 (Gal'-C3), 82.41 (Gal-C3), 99.72, 99.91 (Sia-C2, Sia'-C2), 101.76 (Gal-C1), 104.00 (Gal'-C1), 173.80, 174.64, 174.93, 175.08 (4×CO).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-β-D-galactopyranoside (**81**), (GG-123, *I-124*).

A solution of **41** (100 mg, 96.2 μmol) in MeOH (2.0 mL), dioxane (0.5 mL) and AcOH (0.5 mL) was hydrogenolyzed (4 bar H_2) in the presence of 10% Pd-C (150mg) for 9d. The reaction mixture was filtered through a pad of Celite, which was washed with DCM (2×5 mL). The combined filtrates were concentrated and the residue was purified by silica gel chromatography (PE/AcOEt 2:1) to afford **81** (55.4 mg, 67%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.94-1.00 (m, 2H, $SiCH_2$), 3.47 (t, $J_{5,6a}$=$J_{5,6b}$ =5.6 Hz, 1H, Gal-H5), 3.54 (m, 1H, OCH_2-Ha), 3.65-3.68 (m, 2H, Gal-H3, Gal-H6a), 3.73 (m, 1H, Gal-H2), 3.89 (dd, $J_{5,6b}$=6.6, $J_{6a,6b}$=11.7 Hz, 1H, Gal-H6b), 3.99 (m, 1H, OCH_2-Hb), 4.08 (d, $J_{3,4}$=2.9 Hz, 1H, Gal-H4), 4.22 (d, $J_{1,2}$=7.6 Hz, 1H, Gal-H1), 4.39 (m, 1H, Gal'-H5), 4.48 (dd, $J_{5,6a}$=5.6, $J_{6a,6b}$=11.5 Hz, 1H, Gal'-H6a), 4.68 (dd, $J_{5,6b}$=7.2, $J_{6a,6b}$=11.5 Hz, 1H, Gal'-H6b), 5.16(d, $J_{1,2}$=7.9 Hz, 1H, Gal'-H1), 5.68 (dd, $J_{2,3}$=10.5, $J_{3,4}$=3.5 Hz, 1H, Gal'-H3), 5.81 (dd, $J_{1,2}$=7.9, $J_{2,3}$=10.4 Hz, 1H, Gal'-H2), 6.01 (d, $J_{3,4}$=3.2 Hz, 1H, Gal'-H4), 7.26-7.29, 7.37-7.40, 7.45-7.48, 7.51-7.55, 7.59-7.62, 7.65-7.68, 7.80-7.81, 7.97-7.98, 8.03-8.05, 8.12-8.13 (m, 20H, 4×C_6H_5);

ESI-MS: Calcd for $C_{45}H_{50}O_{15}Si$ $(M+Na)^+$: 881.3; Found: m/z 881.6.

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2-*O*-benzyl-β-D-galactopyranoside (**82**), (GG-121, *I-121*).

A solution of **41** (100 mg, 96.0 μmol) in dioxane (4.0 mL), containing *cat.* AcOH was hydrogenolyzed (4 bar H_2) in the presence of 10% Pd-C (100 mg) for 15h. The

reaction mixture was filtered through a pad of Celite, which was washed with DCM (2×5 mL). The combined filtrates were concentrated, and the residue was purified by silica gel chromatography (PE/AcOEt 1:1) to afford **82** (4.50 mg, 5.0%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ -0.03 (s, 9H, $SiMe_3$), 0.95-0.98 (m, 2H, $SiCH_2$), 3.38 (m, 1H, Gal-H5), 3.50 (m, 1H, OCH_2-Ha), 3.57 (m, 1H, Gal-H2), 3.65 (dd, $J_{5,6a}$ = 4.6, $J_{6a,6b}$ = 11.6 Hz, 1H, Gal-H6a), 3.78 (dd, $J_{2,3}$ = 9.3, $J_{3,4}$ = 3.4 Hz, 1H, Gal-H3), 3.89 (dd, $J_{5,6b}$ = 6.6, $J_{6a,6b}$ = 11.7 Hz, 1H, Gal-H6b), 3.95 (m, 1H, OCH_2-Hb), 4.08 (d, $J_{3,4}$ = 3.3 Hz, 1H, Gal-H4), 4.26-4.35 (m, 3H, PhC$\underline{H}_2$-HA, Gal-H1, Gal'-H5), 4.46 (dd, $J_{5,6a}$ = 5.5, $J_{6a,6b}$ = 11.5 Hz, 1H, Gal'-H6a), 4.64 (dd, $J_{5,6b}$ = 7.2, $J_{6a,6b}$ = 11.5 Hz, 1H, Gal'-H6b), 4.67 (B of AB, J = 10.8 Hz, PhC$\underline{H}_2$-HB), 5.19 (d, $J_{1,2}$ = 8.0 Hz, 1H, Gal'-H1), 5.59 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H3), 5.84 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.4 Hz, 1H, Gal'-H2), 6.01 (d, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H4), 7.09-7.10, 7.22-7.27, 7.41-7.46, 7.50-7.54, 7.57-7.60, 7.63-7.66, 7.75-7.77, 7.84-7.85, 8.00-8.02, 8.10-8.12 (m, 25H, 5×C_6H_5).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (**83**), (GG-130, *I-130*).

To a stirred solution of **42** (82.8 mg, 133 μmol) in py (2.0 mL) at 0°C was added Ac_2O (1.0 mL) and *cat*. DMAP. The mixture was stirred for 16 h at rt under argon, and then quenched by MeOH (1 mL). The solution was concentrated under reduced pressure and co-evaporated with toluene (3×5 mL). The residue was purified by silica gel chromatography (PE/AcOEt 3:1) to afford **83** (101 mg, 92%) as white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (s, 9H, $SiMe_3$), 1.00-1.10 (m, 2H, $SiCH_2$), 1.90, 1.98, 2.01, 2.10, 2.16 (5s, 15H, 5×CH_3-OAc), 3.55 (d, $J_{5,6}$ = 5.9 Hz, 2H, Gal-H6a, Gal-H6b), 3.61 (m, 2H, Gal-H2, OCH_2-Ha), 3.71 (t, $J_{5,6}$ = 5.9 Hz, 1H, Gal-H5), 3.75 (t, $J_{5,6}$ = 6.7 Hz, 1H, Gal'-H5), 3.83 (dd, $J_{2,3}$ = 9.5, $J_{3,4}$ = 3.2 Hz, 1H, Gal-H3), 4.02-4.12 (m, 3H, Gal'-H6a, Gal'-H6b, OCH_2-Hb), 4.41 (d, $J_{1,2}$ = 7.6 Hz, 1H, Gal-H1), 4.49, 4.53 (A, B of AB, J = 11.6 Hz, 2H, PhC$\underline{H}_2$), 4.59 (A of AB, J = 10.3 Hz, 1H, PhC$\underline{H}_2$-HA), 4.86 (d, $J_{1,2}$ = 7.9 Hz, 1H, Gal'-H1), 4.93-4.95 (m, 2H, PhC$\underline{H}_2$-HB, Gal'-H3), 5.16 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$ = 9.9 Hz, 1H, Gal'-H2), 5.33 (d, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H4), 5.39 (d, $J_{3,4}$ = 3.5 Hz, 1H, Gal-H4), 7.26-7.38 (m, 10H, 2×C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.51 (3C, $SiMe_3$), 18.41 ($SiCH_2$), 20.59, 20.61, 20.68, 20.76 (5C, 5×CH_3-OAc), 61.07 (Gal'-C6), 66.94 (Gal'-C4), 67.88 (OCH_2), 68.94 (Gal-C6), 69.14 (Gal'-C2), 69.58 (Gal-C4), 70.67 (Gal'-C5), 70.76 (Gal-C3), 72.77 (Gal-C5), 73.73 (Ph<u>C</u>H_2), 75.21 (Ph<u>C</u>H_2), 79.79 (Gal-C2), 100.71 (Gal'-C1), 103.11 (Gal-C1), 127.88, 128.06, 128.43, 128.54 (12C, 2×C_6H_5).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-β-D-galactopyranoside (**84**), (GG-131, *I-131*).

A solution of **83** (100 mg, 0.120 mmol) in MeOH (4.0 mL), dioxane (1.0 mL) and AcOH (1.0 mL) was hydrogenolyzed (4 bar H_2) in the presence of 10% Pd-C (150 mg) for 2d. The reaction mixture was filtered through a pad of Celite, which was washed with DCM (2×5 mL). The combined filtrates were concentrated, and the residue was purified by silica gel chromatography (PE/AcOEt 1:1) to afford **84** (73.9 mg, 94%) as white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (s, 9H, $SiMe_3$), 0.94-1.08 (m, 2H, $SiCH_2$), 1.99, 2.06, 2.15, 2.16 (4s, 15H, 5×CH_3-OAc), 3.43 (dd, $J_{5,6}$ = 7.8, 11.1 Hz, 1H, Gal-H5), 3.56-3.68 (m, 3H, Gal-H6a, Gal-H6b, OCH_2-Ha), 3.71 (dd, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.4 Hz, 1H, Gal-H3), 3.77 (m, 1H, Gal-H2), 3.90 (t, $J_{5,6}$ = 6.6 Hz, 1H, Gal'-H5), 4.00 (m, 1H, OCH_2-Hb), 4.08 (d, $J_{5,6}$ = 6.6 Hz, 2H, Gal'-H6a, Gal'-H6b), 4.30 (d, $J_{1,2}$ = 7.5 Hz, 1H, Gal-H1), 4.72 (d, $J_{1,2}$ = 7.9 Hz, 1H, Gal'-H1), 5.02 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 3.4 Hz, Gal'-H3), 5.19 (dd, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.5 Hz, 1H, Gal'-H2), 5.26 (d, $J_{3,4}$ = 3.4, 1H, Gal-H4), 5.37 (d, $J_{3,4}$ = 3.5, 1H, Gal'-H4);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.46 (3C, $SiMe_3$), 18.25 ($SiCH_2$), 20.58, 20.65, 20.80, 20.82 (5C, 5×CH_3-OAc), 59.86 (Gal-C5), 61.09 (Gal'-C6), 66.78 (Gal'-C4), 67.80 (OCH_2), 68.92 (Gal-C6), 70.48 (2C, Gal-C4, Gal'-C2), 70.78 (Gal'-C3), 73.32 (2C, Gal-C3, Gal'-C5), 79.91 (Gal-C2), 101.58 (Gal-C1), 102.48 (Gal'-C1), 169.90, 170.10, 170.19, 170.44 (5C, 5×CO).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*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)-β-D-galactopyranoside (**85**), (GG-129, *I-129*).

To a solution of **81** (50.0 mg, 58.2 μmol) and **55** (60.7 mg, 0.12 mmol) in CH_3CN/DCM (3:1, 4 mL) was added activated powdered molecular sieves 3Å (1.00

g). The suspension was stirred at rt under argon for 6 h, then cooled to –25°C. After addition of NIS (52.4 mg, 0.233 mmol) and TfOH (2.0 μL, 23.3 μmol), stirring was continued for 16 h at –25°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% aq. $Na_2S_2O_3$ (30 mL), 5% $KHCO_3$ (2×20 mL) and H_2O (20 mL), dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH 60:1) to afford **85** (35.0 mg, 45%) as white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.95-1.01 (m, 2H, $SiCH_2$), 1.89, 2.02, 2.03, 2.12, 2.15 (5s, 15H, CH_3-NHAc, 4×CH_3-OAc), 1.94 (m, 1H, Sia-H3a), 2.59 (dd, $J_{3a,3b}$ = 12.8, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.51-3.58 (m, 2H, Gal-H5, OCH_2-Ha), 3.65-3.68 (m, 2H, Gal-H2, Gal-H6a), 3.74 (dd, $J_{2,3}$ = 9.4, $J_{3,4}$ = 3.2 Hz, 1H, Gal-H3), 3.81 (s, 3H, OCH_3), 3.88 (dd, $J_{5,6b}$ = 5.5, $J_{6a,6b}$ = 9.6 Hz, 1H, Gal-H6b), 3.98 (m, 1H, OCH_2-Hb), 4.06-4.13 (m, 4H, Gal-H4, Sia-H5, Sia-H6, Sia-H9a), 4.19 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal-H1), 4.30-4.38 (m, 2H, Gal'-H5, Sia-H9b), 4.42 (dd, $J_{5,6a}$ = 7.0, $J_{6a,6b}$ = 11.1 Hz, 1H, Gal'-H6a), 4.70 (dd, $J_{5,6b}$ = 6.2, $J_{6a,6b}$ = 11.1 Hz, 1H, Gal'-H6b), 4.86 (ddd, $J_{3b,4}$ = 4.6, $J_{3a,4}$ = 12.2, $J_{4,5}$ = 9.8 Hz, 1H, Sia-H4), 5.24 (d, $J_{5,NH}$ = 9.6 Hz, 1H, Sia-NH), 5.30-5.34 (m, 2H, Gal'-H1, Sia-H7), 5.36-5.40 (m, 1H, Sia-H8), 5.66 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.5 Hz, 1H, Gal'-H3), 5.80 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.4 Hz, 1H, Gal'-H2), 6.02 (d, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H4), 7.24-7.27, 7.36-7.39, 7.43-7.47, 7.50-7.55, 7.57-7.60, 7.63-7.66, 7.78-7.80, 7.95-7.97, 8.01-8.03, 8.10-8.12(m, 20H, 4×C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.44 (3C, $SiMe_3$), 18.05 ($SiCH_2$), 20.75, 20.81, 20.99 (4C, 4×CH_3-OAc), 23.16 (CH_3-NHAc), 37.56 (Sia-C3), 49.39 (Sia-C5), 52.91 (OCH_3), 61.68 (Gal'-C6), 62.41 (Sia-C9), 62.61 (Gal-C6), 67.08 (OCH_2), 67.47 (2C, Gal-C4, Sia-C7), 67.64 (Gal'-C4), 67.95 (2C, Sia-C4, Sia-C8), 68.90 (Gal'-C2), 70.01 (Gal-C2), 71.13, 71.36 (Gal'-C5, Gal'-C3), 72.30 (Sia-C6), 72.80 (Gal-C5), 81.17 (Gal-C3), 98.96 (Sia-C2), 101.91, 102.10 (Gal-C1, Gal'-C1), 128.27, 128.42, 128.51, 128.64, 128.69, 128.97, 129.23, 129.29, 129.67, 129.76, 130.01, 130.99, 133.30, 133.36, 133.63 (24C, 4×C_6H_5), 165.50, 165.55, 165.61, 169.02, 169.88, 170.10, 170.27, 170.68, 170.94 (10C, 10×CO);

HRMS (FAB) Calcd for $C_{66}H_{77}NO_{27}Si$ $(M+NH_4)^+$: 1349.4796; Found: m/z 1349.4778.

2-(Trimethylsilyl)ethyl β-D-galactopyranosyl-(1→3)-[(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)]-(2→6)-β-D-galactopyranoside (**45**), (GG-136, *I-136*).

To a solution of **85** (32.5 mg, 24.4 μmol) in MeOH (1 mL) was added freshly prepared 1M NaOMe/MeOH (1.0 mL). The mixture was stirred at rt under argon for 7h, then H_2O (1 mL) was added and the mixture was stirred for 16 hr at rt. The solution was concentrated and the residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **45** as white solid (17.8 mg, 90%) after a final lyophilization from H_2O.

$[\alpha]_D$ -3.5° (*c* = 0.54, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.00 (s, 9H, $SiMe_3$), 0.95 (td, *J* = 12.9, *J* = 5.2 Hz, 1H, $SiCH_2$-Ha), 1.04 (td, *J* = 12.9, *J* = 5.5 Hz, 1H, $SiCH_2$-Hb), 1.63 (t, $J_{3a,3b} = J_{3a,4} = 12.2$ Hz, 1H, Sia-H3a), 2.00 (s, 3H, CH_3-NHAc), 2.68 (dd, $J_{3a,3b} = 12.4$, $J_{3b,4} = 4.7$ Hz, 1H, Sia-H3b), 3.55 (m, 1H, Sia-H6), 3.56-3.60 (m, 3H, Gal-H2, Gal'-H2, Sia-H9a), 3.61-3.64 (m, 2H, Gal-H6a, Gal'-H3), 3.66-3.68 (m, 3H, Gal'-H5, Sia-H4, Sia-H7), 3.71 (m, 1H, Gal'-H6a), 3.72-3.79 (m, 4H, Gal-H3, Gal'-H6b, Sia-H8, OCH_2-Ha), 3.81-3.85 (m, 3H, Gal-H5, Gal-H6b, Sia-H5), 3.87-3.91 (m, 2H, Gal'-H4, Sia-H9b), 4.00 (ddd, *J* = 5.1, *J* = 10.1, *J* = 12.7 Hz, 1H, OCH_2-Hb), 4.20 (d, $J_{3,4} = 3.3$ Hz, 1H, Gal-H4), 4.42 (d, $J_{1,2} = 8.0$ Hz, 1H, Gal-H1), 4.55 (d, $J_{1,2} = 7.6$ Hz, 1H, Gal'-H1);

^{13}C NMR (125 MHz, D_2O): δ 0.00 (3C, $SiMe_3$), 19.28 ($SiCH_2$), 42.14 (Sia-C3), 54.36 (Sia-C5), 63.59 (Gal'-C6), 65.19 (Gal-C6), 65.64 (Sia-C9), 70.67, 70.72 (Gal'-C5, Sia-C6), 70.68 (OCH_2), 71.10 (2C, Gal-C4, Gal'-C4), 72.20 (Gal-C2), 73.50 (Gal'-C2, Sia-C7), 74.17 (Gal-C5), 75.14 (Sia-C4), 75.48 (Sia-C8), 77.57 (Gal'-C3), 85.18 (Gal-C3), 102.41 (Sia-C2), 104.27 (Gal-C1), 106.97 (Gal'-C1), 175.43, 177.57 (2×CO);

ESI-MS: Calcd for $C_{28}H_{50}NNaO_{19}Si$ $(M+Na)^+$: 778.3; Found: m/z 778.5.

2-(Trimethylsilyl)ethyl 2,6-*O*-di(*p*-methoxybenzyl)-3,4-*O*-isopropylidene-β-D-galactopyranoside (**90**), (JPB-11).

To a solution of **39** (1.00 g, 3.12 mmol) in DMF (30 mL) at 0°C were added NaH (60% in oil, 300 mg, 12.5 mmol) and a catalytic amount of TBAI. MPMCl (1.69 mL, 12.5 mmol) was added dropwise during 10 min under argon and the mixture was stirred at 0°C for 3 h. The reaction was quenched by adding MeOH, then diluted with

DCM (70 mL), and washed with 5% $NaHCO_3$ (2×50 mL) and H_2O (2×50 mL). The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by silica gel chromatography (1% gradient AcOEt in toluene, start from 5%) to afford **90** (1.07g, 61%) as white foam.

$[\alpha]_D$ +18.3° (*c* = 0.49, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (s, 9H, $SiMe_3$), 1.03-1.07 (m, 2H, $SiCH_2$), 1.31, 1.34 (2s, 6H, 2×CH_3), 3.35 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$ = 6.3, 1H, Gal-H2), 3.57 (m, 1H, OCH_2-Ha), 3.74-3.76 (m, 2H, Gal-H6a, Gal-H6b), 3.78, 3.79 (2s, 6H, 2×OCH_3-MPM), 3.93 (ddd, $J_{4,5}$ = 1.6, $J_{5,6a}$ = 5.3, $J_{5,6b}$ = 6.9 Hz, 1H, Gal-H5), 4.03 (m, 1H, OCH_2-Hb), 4.09-4.13 (m, 2H, Gal-H3, Gal-H4), 4.29 (d, $J_{1,2}$ = 8.1 Hz, 1H, Gal-H1), 4.48, 4.55 (A, B of AB, *J* = 11.4 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.72, 4.76 (A, B of AB, *J* = 11.3 Hz, 2H, $C_6H_4C\underline{H}_2$), 6.84-6.87, 7.25-7.32 (m, 8H, 2×C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.43 (3C, $SiMe_3$), 18.45 ($SiCH_2$), 26.39, 27.78 (2×CH_3), 55.26 (2C, 2×OCH_3-MPM), 67.16 (OCH_2), 69.37 (Gal-C6), 72.19 (Gal-C5), 73.23, 73.27 (2×$C_6H_4\underline{C}H_2$), 73.87 (Gal-C4), 79.12 (Gal-C3), 79.34 (Gal-C2), 102.44 (Gal-C1), 109.81 ($\underline{C}Me_2$), 113.59, 113.78, 129.25, 129.83, 130.33, 130.56, 159.13, 159.21 (12C, 2×C_6H_4).

2-(Trimethylsilyl)ethyl 2,6-*O*-di(*p*-methoxybenzyl)-β-D-galactopyranoside (**91**), (JPB-14).

90 (1.00 g, 1.78 mmol) was suspended in 80% aq. AcOH (30 mL) and stirred at 90°C for 1.5 h. The reaction was concentrated under reduced pressure and the residue was purified by flash silica gel chromatography (15% gradient AcOEt in toluene, start from 30%) to afford **91** (460 mg, 50%) as white foam.

$[\alpha]_D$ +8.3° (*c* = 0.53, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.04 (s, 9H, $SiMe_3$), 1.04-1.07 (m, 2H, $SiCH_2$), 3.46 (dd, $J_{1,2}$ = 7.7, $J_{2,3}$ = 9.4 Hz, 1H, Gal-H2), 3.55 (dd, $J_{2,3}$ = 9.4, $J_{3,4}$ = 3.2 Hz, 1H, Gal-H3), 3.57-3.62 (m, 2H, Gal-H5, OCH_2-Ha), 3.71 (dd, $J_{5,6a}$ = 5.7, $J_{6a,6b}$ = 10.1 Hz, 1H, Gal-H6a), 3.76 (dd, $J_{5,6b}$ = 5.6, $J_{6a,6b}$ = 10.1 Hz, 1H, Gal-H6b), 3.79, 3.80 (2s, 6H, 2×OCH_3-MPM), 3.97 (d, $J_{3,4}$ = 3.0 Hz, 1H, Gal-H4), 4.04 (m, 1H, OCH_2-Hb), 4.36 (d, $J_{1,2}$ = 7.8 Hz, 1H, Gal-H1), 4.51 (s, 2H, $C_6H_4C\underline{H}_2$), 4.62, 4.89 (A, B of AB, *J* = 11.2 Hz, 2H, $C_6H_4C\underline{H}_2$), 6.86-6.89, 7.25-7.31 (m, 8H, 2×C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.42 (3C, $SiMe_3$), 18.53 ($SiCH_2$), 55.26 (2C, 2×OCH_3-MPM), 67.33 (OCH_2), 69.05 (2C, Gal-C4, Gal-C6), 73.22 (Gal-C5), 73.33,

74.11 (2×$C_6H_4\underline{C}H_2$), 78.62 (2C, Gal-C2, Gal-C3), 103.19 (Gal-C1), 113.84, 113.95, 129.33, 129.82, 129.95, 130.54, 159.29, 159.36 (12C, 2×C_6H_4).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-*O*-di-(*p*-methoxybenzyl)-β-D-galactopyranoside (**92**), (GG-269, *I-272*).

A mixture of **91** (307 mg, 0.590 mmol), **30** (567 mg, 0.885 mmol) and activated powdered molecular sieves 3Å (2.00 g) in DCM (10 mL) was stirred at rt for 6h under argon. DMTST (685 mg, 2.65 mmol) was then added at 0°C. The reaction mixture was stirred at 0°C for 16h, then 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 5% $NaHCO_3$ (2×20 mL) and H_2O (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (1% gradient AcOEt in toluene, start from 4%) to afford **92** (491 mg, 76%) as white foam.

$[\alpha]_D$ +54.1° (*c* = 0.58, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (s, 9H, $SiMe_3$), 0.93-1.00 (m, 2H, $SiCH_2$), 3.54-3.60 (m, 3H, Gal-H2, Gal-H5, OCH_2-Ha), 3.68-3.74 (m, 2H, Gal-H6a, Gal-H6b), 3.77-3.87 (m, 7H, Gal-H3, 2×OCH_3-MPM), 3.99-4.04 (m, 1H, OCH_2-Hb), 4.12 (d, $J_{3,4}$ = 3.1 Hz, 1H, Gal-H4), 4.27 (m, 1H, Gal-H5), 4.30, 4.66 (A, B of AB, *J* = 10.1 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.32 (d, $J_{1,2}$ = 7.8 Hz, 1H, Gal-H1), 4.46 (dd, $J_{5,6a}$ = 6.2, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal'-H6a), 4.48, 4.52 (A, B of AB, *J* = 11.5 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.63 (m, 1H, Gal'-H6b), 5.28 (d, $J_{1,2}$ = 8.0 Hz, 1H, Gal'-H1), 5.65 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 3.5 Hz, 1H, Gal'-H3), 5.87 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.4 Hz, 1H, Gal'-H2), 6.00 (d, $J_{3,4}$ = 3.4 Hz, 1H, Gal'-H4), 6.90-6.92, 7.10-7.12, 7.26-7.32, 7.44-7.49, 7.54-7.60, 7.66-7.69, 7.80-7.81, 7.91-7.92, 8.03-8.05, 8.13-8.15 (m, 28H, 4×C_6H_5, 2×C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.50 (3C, $SiMe_3$), 18.47 ($SiCH_2$), 55.25 (2C, 2×OCH_3-MPM), 61.96 (Gal'-C6), 67.22 (OCH_2), 68.13 (Gal'-C4), 68.86 (Gal-C4), 69.14 (Gal-C6), 69.93 (Gal'-C2), 71.42 (Gal'-C5), 71.49 (Gal'-C3), 73.13 (Gal-C5), 73.26, 74.40 (2C, 2×$C_6H_4\underline{C}H_2$), 78.59 (Gal-C2), 80.70 (Gal-C3), 101.53 (Gal'-C1), 102.95 (Gal-C1), 113.79, 113.81, 128.41, 128.49, 128.69, 128.93, 129.25, 129.30, 129.35, 129.68, 129.74, 129.78, 130.04, 130.23, 130.63, 133.35, 133.67, 159.03, 159.22 (36C, 4×C_6H_5, 2×C_6H_4), 165.508, 165.59, 165.91 (4C, 4×CO).

2-(Trimethylsilyl)ethyl (β-D-galactopyranosyl)-(1→3)-2,6-*O*-di-(*p*-methoxybenzyl)-β-D-galactopyranoside (**93**), (GG-273, *I-273*).

To a solution of **92** (491 mg, 0.447 mmol) in MeOH (20 mL) was added freshly prepared 1M NaOMe/MeOH (2.0 mL). The mixture was stirred at rt for 2h under argon, then neutralized with Amberlite IRC 50 ion-exchange resin and filtered through a pad of Celite. The Celite was washed with MeOH (3×10 mL) and the combined filtrates were evaporated under reduced pressure. The residue was purified by flash silica gel chromatography (2% gradient MeOH in DCM) to afford **93** (260 mg, 85%) as white foam.

$[\alpha]_D$ -7.7°, (*c* = 0.82, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 0.03 (s, 9H, $SiMe_3$), 1.01 (t, *J* = 8.4 Hz, 2H, CH_2Si), 3.43 (m, 1H, Gal-H5), 3.46 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H3), 3.57-3.72 (m, 8H, Gal-H2, Gal-H6a, Gal-H6b, Gal'-H2, Gal'-H5, Gal'-H6a, Gal'-H6b, OCH_2-Ha), 3.75 (m, 1H, Gal-H3), 3.76, 3.77 (2s, 6H, 2×OCH_3), 3.81 (d, $J_{3,4}$ = 3.0 Hz, 1H, Gal'-H4), 3.98-4.03 (m, 2H, Gal-H4, OCH_2-Hb), 4.35 (d, $J_{1,2}$ = 7.8 Hz, 1H, Gal-H1), 4.47, 4.51 (A, B of AB, *J* = 11.4 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.59 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal'-H1), 4.73, 4.76 (A, B of AB, *J* = 10.1 Hz, 2H, $C_6H_4C\underline{H}_2$), 6.85-6.89, 7.25-7.27, 7.35-7.37 (m, 8H, 2×C_6H_4);

^{13}C NMR (125 MHz, CD_3OD): δ -1.32 (3C, $SiMe_3$), 19.45 ($SiCH_2$), 55.65, 55.67 (2×OCH_3), 62.54 (Gal'-C6), 68.40 (OCH_2), 70.28 (Gal'-C4), 70.52 (Gal-C6), 70.63 (Gal-C4), 72.80 (Gal'-C2), 74.08, ($C_6H_4\underline{C}H_2$), 74.66 (Gal-C5), 74.76 (Gal-C3), 75.67 ($C_6H_4\underline{C}H_2$), 76.66 (Gal-C5), 80.12 (Gal-C2), 81.82 (Gal-C3), 104.46 (Gal-C1), 105.94 (Gal'-C1), 114.53, 114.74, 130.48, 131.17, 131.58, 132.26, 160.75, 160.83 (12C, 2×C_6H_4);

Anal. Calcd for $C_{33}H_{50}O_{13}Si$: C, 58.05; H, 7.38. Found: C, 58.07; H, 7.28.

2-(Trimethylsilyl)ethyl (6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-*O*-di-(*p*-methoxybenzyl)-β-D-galactopyranoside (**89**), (GG-275, *I-276*).

To a solution of **93** (260 mg, 0.381 mmol) in Et_3N (4.0 mL) and CH_3CN (16 mL) at –40°C under argon, a solution of benzoyl cyanide (45.0 mg, 0.343 mmol) in CH_3CN (0.5 mL) was added dropwise during 15 min. The reaction was stirred at –40~-45°C for 1h and then quenched by a few drops of MeOH. The solution was concentrated

under reduced pressure and the residue was purified by silica gel chromatography (1% gradient MeOH in DCM) to afford **89** (180 mg, 60%) as white foam.

$[\alpha]_D$ -3.1°, (*c* = 0.82, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 0.03 (s, 9H, $SiMe_3$), 0.98-1.01 (m, 2H, $SiCH_2$), 3.42-3.46 (m, 2H, Gal-H5, Gal-H6a), 3.53 (dd, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.4Hz, 1H, Gal'-H3), 3.55-3.65 (m, 5H, Gal-H2, Gal-H3, Gal-H6b, Gal'-H2, OCH_2-Ha), 3.76, 3.77 (2s, 6H, 2×OCH_3), 3.83 (dd, $J_{5,6a}$ = 4.5, $J_{5,6b}$ = 8.5 Hz, 1H, Gal'-H5), 3.88 (d, $J_{3,4}$ = 3.3 Hz, 1H, Gal'-H4), 3.96-3.99 (m, 2H, Gal-H4, OCH_2-Hb), 4.25 (d, $J_{1,2}$ = 7.4 Hz, 1H, Gal-H1), 4.37-4.41 (m, 3H, Gal'-H6a, $C_6H_4C\underline{H}_2$), 4.58 (d, $J_{1,2}$ = 7.7 Hz, 1H, Gal'-H1), 4.63 (dd, $J_{5,6b}$ = 8.4, $J_{6a,6b}$ = 11.4 Hz, 1H, Gal'-H6b), 4.71, 4.77 (A, B of AB, *J* = 10.1 Hz, 2H, $C_6H_4C\underline{H}_2$), 6.85-6.87, 7.21-7.23, 7.35-7.36, 7.44-7.47, 7.56-7.59, 7.83-8.02 (m, 13H, C_6H_5, 2× C_6H_4);

^{13}C NMR (125 MHz, CD_3OD): δ -1.46 (3C, $SiMe_3$), 19.28 ($SiCH_2$), 55.51, 55.54 (2×OCH_3), 66.01 (Gal'-C6), 68.14 (OCH_2), 70.13 (Gal'-C4), 70.51 (Gal-C6), 70.59 (Gal-C4), 72.44 (Gal'-C2), 73.84 ($C_6H_4\underline{C}H_2$), 73.93 (Gal'-C5), 74.50 (Gal-C5), 74.57 (Gal'-C3), 75.50 ($C_6H_4\underline{C}H_2$), 79.67 (Gal-C2), 82.65 (Gal-C3), 104.21 (Gal-C1), 105.88 (Gal'-C1), 114.40, 114.59, 129.58, 130.23, 130.51, 130.83, 131.46, 133.08, 134.28 (18C, C_6H_5, 2×C_6H_4), 167.56 (CO);

Anal. Calcd for $C_{40}H_{54}O_{14}Si$: C, 61.05; H, 6.92. Found: C, 60.57; H, 6.70.

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-9-azido-4,7,8-tri-*O*-acetyl-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,6-*O*-di-(*p*-methoxybenzyl)-β-D-galactopyranoside (**94**), (GG-281, *I-282*).

A mixture of **89** (77.0 mg, 97.8 μmol), methyl (methyl 5-acetamido-9-azido-4,7,8-tri-*O*-acetyl-3,5,9-trideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[178] **88** (98.0 mg, 196 μmol) and activated powdered molecular sieves 3Å (1.00 g) was stirred in CH_3CN (5 mL) at rt for 6 h under argon. DMTST (202 mg, 0.783 mmol) was then added at 0°C. The reaction mixture was stirred at 0°C for 16 h, diluted with DCM (5 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3×5 mL), and the combined filtrates were washed with 5% $NaHCO_3$ (2×10 mL) and H_2O (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0.25%

gradient MeOH in DCM) to afford **94** (90.5 mg, 47%) as white foam (β-isomer: 30.0 mg, 16%).

$[\alpha]_D$ -3.3°, (*c* = 0.91, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.01 (s, 9H, $SiMe_3$), 1.01-1.04 (m, 2H, $SiCH_2$), 1.90 (s, 3H, CH_3-NHAc), 2.04, 2.10, 2.13 (3s, 9H, 3×CH_3-OAc), 2.11 (m, 1H, Sia-H3a), 2.69 (dd, $J_{3a,3b}$ = 13.1, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.11 (dd, $J_{8,9a}$ = 5.4, $J_{9a,9b}$ = 13.6 Hz, 1H, Sia-H9a), 3.44 (dd, $J_{8,9b}$ = 2.6, $J_{9a,9b}$ = 13.6 Hz, 1H, Sia-H9b), 3.49-3.59 (m, 3H, Gal-H5, Gal-H6a, OCH_2-Ha), 3.61-3.71 (m, 4H, Gal-H2, Gal-H3, Gal-H6b, Gal'-H4), 3.75-3.81 (m, 1H, Gal'-H2), 3.77, 3.78 (2s, 6H, 2×OCH_3-MPM), 3.80 (s, 3H, OCH_3), 3.84 (m, 1H, Gal'-H5), 4.00 (d, $J_{3,4}$ = 3.2 Hz, 1H, Gal-H4), 4.01-4.13 (m, 4H, Gal'-H3, Sia-H5, Sia-H6, OCH_2-Hb), 4.32(d, $J_{1,2}$ = 7.5 Hz, 1H, Gal-H1), 4.39, 4.43 (A, B of AB, *J* = 11.5 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.50 (dd, $J_{5,6a}$ = 7.6, $J_{6a,6b}$ = 11.6 Hz, 1H, Gal'-H6a), 4.60 (d, $J_{1,2}$ = 7.8Hz, 1H, Gal'-H1), 4.61 (m, 1H, Gal'-H6b), 4.75, 4.78 (A, B of AB, ^2J = 10.7 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.94 (ddd, $J_{3b,4}$ = 4.6, $J_{3a,4}$ = 12.0, $J_{4,5}$ = 10.0 Hz, 1H, Sia-H4), 5.24(d, $J_{5,NH}$ = 9.5 Hz, 1H, Sia-NH), 5.28-5.34 (m, 2H, Sia-H7, Sia-H8), 6.82-6.86, 7.21-7.23, 7.34-7.36, 7.39-7.42, 7.53-7.56, 8.01-8.03 (m, 13H, C_6H_5, 2× C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.50 (3C, $SiMe_3$), 18.41 ($SiCH_2$), 20.72, 20.76, 21.06 (3×CH_3-OAc), 22.13 (CH_3-NHAc), 37.42 (Sia-C3), 49.38 (Sia-C5), 50.98 (Sia-C9), 53.15 ($CO_2\underline{C}H_3$), 55.15, 55.19 (2×O$\underline{C}H_3$-MPM), 63.21 (Gal'-C6), 67.10 (OCH_2), 67.55, 67.57 (Gal'-C4, Sia-C7), 68.45 (Sia-C4), 68.64 (Gal-C4), 68.86 (Sia-C8), 69.30 (Gal'-C2), 69.50 (Gal-C6), 72.07 (Gal'-C5), 72.81 (Sia-C6), 73.12 ($C_6H_4\underline{C}H_2$), 73.18 (Gal-C5), 74.45 ($C_6H_4\underline{C}H_2$), 76.08 (Gal'-C3), 77.52 (Gal-C2), 84.00 (Gal-C3), 97.54 (Sia-C2), 102.83 (Gal-C1), 103.77 (Gal'-C1), 113.55, 113.73, 128.42, 129.20, 129.57, 129.62, 129.84, 130.27, 133.27, 159.11 (18C, C_6H_5, 2× C_6H_4), 166.18, 168.35, 170.02, 170.08, 170.30, 170.87 (6×CO);

Anal. Calcd for $C_{58}H_{78}N_4O_{24}Si$: C, 56.03; H, 6.32; N, 4.51. Found: C, 55.81; H, 6.32; N, 4.35.

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-9-azido-4,7,8-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-(2,4-*O*-diacetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-2,6-*O*-di-(*p*-methoxybenzyl)-β-D-galactopyranoside (**95**), (GG-284, *I-284*).

To a stirred solution of **94** (87.0 mg, 70.0 μmol) in py (0.4 mL) at 0°C was added Ac_2O (0.2 mL). The mixture was stirred at rt for 16 h under argon, and then quenched by MeOH (1 mL). The solution was concentrated and co-evaporated with toluene (3×5 mL), and the residue was purified by silica gel chromatography (10% gradient AcOEt in toulene) to afford **95** (83.0 mg, 87%) as white foam.

$[\alpha]_D$ -8.6°, (*c* = 0.66, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.99-1.04 (m, 2H, $SiCH_2$), 1.67 (t, $J_{3a,3b} = J_{3a,4} = 12.5$ Hz, 1H, Sia-H3a), 1.84 (s, 3H, CH_3-NHAc), 1.92, 2.00, 2.02, 2.09, 2.11, 2.15 (6s, 18H, 6×CH_3-OAc), 2.58 (dd, $J_{3a,3b}$ = 12.7, $J_{3b,4} = 4.6$ Hz, 1H, Sia-H3b), 3.09 (dd, $J_{8,9a} = 5.8$, $J_{9a,9b} = 13.6$ Hz, 1H, Sia-H9a), 3.44-3.65 (m, 7H, Gal-H2, Gal-H5, Gal-H6a, Gal-H6b, Sia-H6, Sia-H9b, OCH_2-Ha), 3.73 (s, 3H, CO_2CH_3), 3.78, 3.79 (2s, 6H, 2×OCH_3-MPM), 3.86 (m, 1H, Gal-H3), 3.98-4.05 (m, 3H, Gal'-H5, Sia-H5, OCH_2-Hb), 4.16 (dd, $J_{5,6a} = 7.9$, $J_{6a,6b} = 11.0$ Hz, 1H, Gal'-H6a), 4.36 (d, $J_{1,2} = 7.8$ Hz, 1H, Gal-H1), 4.38-4.44 (m, 3H, Gal'-H6b, $C_6H_4C\underline{H}_2$), 4.54 (dd, $J_{2,3} = 10.2$, $J_{3,4} = 3.3$ Hz, 1H, Gal'-H3), 4.65, 4.76 (A, B of AB, *J* = 10.6 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.83 (d, $J_{1,2} = 8.0$ Hz, 1H, Gal'-H1), 4.85 (m, 1H, Sia-H4), 5.05 (d, $J_{3,4} = 3.3$ Hz, 1H, Gal'-H4), 5.07 (d, $J_{5,NH} = 10.4$ Hz, 1H, Sia-NH), 5.11 (dd, $J_{1,2} = 8.0$, $J_{2,3} = 10.2$ Hz, 1H, Gal'-H2), 5.33 (dd, $J_{6,7} = 2.6$, $J_{7,8} = 8.7$ Hz, 1H, Sia-H7), 5.40-5.43 (m, 2H, Gal-H4, Sia-H8), 6.84-6.87, 7.23-7.25, 7.32-7.33, 7.40-7.43, 7.53-7.56, 8.02-8.04 (m, 13H, C_6H_5, 2×C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.56 (3C, $SiMe_3$), 18.44 ($SiCH_2$), 20.65, 20.69, 20.81, 20.89, 21.18 (6C, 6×CH_3-OAc), 23.08 (CH_3-NHAc), 36.93 (Sia-C3), 48.99 (Sia-C5), 51.18 (Sia-C9), 53.03 ($CO_2\underline{C}H_3$), 55.18 (2C, 2×OCH_3-MPM), 61.15 (Gal'-C6), 67.15 (Gal'-C4), 67.70 (2C, Sia-C7, OCH_2), 68.63, 69.13 (Gal-C4, Sia-C8), 69.19 (Gal-C6), 69.46 (Sia-C4), 70.15 (Gal'-C2), 70.38 (Gal'-C5), 71.48 (Gal'-C3), 72.19 (Sia-C6), 72.93 (Gal-C5), 73.24, 74.69 (2×$C_6H_4\underline{C}H_2$), 78.31 (Gal-C2), 80.17 (Gal-C3), 96.67 (Sia-C2), 100.96 (Gal'-C1), 103.03 (Gal-C1), 113.49, 113.73, 128.23, 129.23, 129.34, 129.62, 129.74, 133.04, 130.05, 131.05, 158.99, 159.15 (18C, C_6H_5, 2×C_6H_4), 165.56, 167.86, 169.66, 170.15, 170.22, 170.39 (9C, 9×CO).

2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzoamido-3,5,9-trideoxy-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-*O*-di-(*p*-methoxybenzyl)-β-D-galactopyranoside (**96**), (GG-286, *I-286*).

95 (79.3 mg, 57.9 μmol) and benzoylchloride (27.0 μL, 0.232 mmol) were dissolved in DCE (5 mL) and stirred at rt under argon. Ph_3P (33.0 mg, 0.127 mmol) in DCE (1 mL) was added after 5 min. The reaction was stirred at rt for 16 h, then diluted with DCM (10 mL) and washed with 5% $NaHCO_3$ (2×10 mL) and H_2O (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0.25% gradient MeOH in DCM) to afford **96** (47.5 mg, 57%) as white foam.

$[\alpha]_D$: +2.3°, (*c* = 0.90, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 9H, $SiMe_3$), 0.98-1.07 (m, 2H, $SiCH_2$), 1.70 (t, $J_{3a,3b} = J_{3a,4} = 12.4$ Hz, 1H, Sia-H3a), 1.84 (s, 3H, CH_3-NHAc), 1.95, 2.00, 2.08, 2.09, 2.10 (6s, 18H, 6×CH_3-OAc), 2.58 (dd, $J_{3a,3b} = 12.7$, $J_{3b,4} = 4.6$ Hz, 1H, Sia-H3b), 2.82 (dt, $J_{9a,9b} = 15.0$, $J_{8,9a} = J_{9a,NH} = 3.9$ Hz, 1H, Sia-H9a), 3.42-3.49 (m, 2H, Gal-H6a, Gal-H6b), 3.53 (dd, $J_{5,6} = 10.8$, $J_{6,7} = 2.6$ Hz, 1H, Sia-H6), 3.59-3.63 (m, 3H, Gal-H2, Gal-H5, OCH_2-Ha), 3.71 (s, 3H, CO_2CH_3), 3.77, 3.78 (2s, 6H, 2×OCH_3-MPM), 4.00-4.05 (m, 2H, Gal'-H5, OCH_2-Hb), 4.11 (q, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.4$ Hz, 1H, Sia-H5), 4.18 (dd, $J_{5,6a} = 7.6$, $J_{6a,6b} = 11.0$ Hz, 1H, Gal'-H6a), 4.31-4.38 (m, 2H, Gal'-H6b, Sia-H9b), 4.36 (d, $J_{1,2} = 7.8$ Hz, 1H, Gal-H1), 4.39, 4.42 (A, B of AB, *J* = 11.2 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.66, 4.76 (A, B of AB, *J* = 10.3 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.67 (m, 1H, Gal'-H3), 4.85 (ddd, $J_{3a,4} = 12.2$, $J_{3b,4} = 4.6$, $J_{4,5} = 10.5$ Hz, 1H, Sia-H4), 4.91 (d, $J_{1,2} = 7.9$ Hz, 1H, Gal'-H1), 5.07 (d, $J_{3,4} = 2.7$ Hz, 1H, Gal'-H4), 5.10 (dd, $J_{1,2} = 7.9$, $J_{2,3} = 10.1$ Hz, 1H, Gal'-H2), 5.13 (d, $J_{5,NH} = 10.4$ Hz, 1H, Sia-NH), 5.20 (dd, $J_{6,7} = 2.6$, $J_{7,8} = 9.2$ Hz, 1H, Sia-H7), 5.34 (dt, $J_{7,8} = 9.2$, $J_{8,9a} = J_{8,9b} = 3.2$ Hz, 1H, Sia-H8), 5.39 (d, $J_{3,4} = 3.6$ Hz, 1H, Gal-H4), 6.82-6.88, 7.23-7.26, 7.31-7.34, 7.39-7.55, 7.77-7.79, 8.01-8.03 (m, 18H, 2×C_6H_5, 2×C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ -1.52 (3C, $SiMe_3$), 18.49 ($SiCH_2$), 20.71, 20.91, 20.93, 21.28 (6C, 6×CH_3-OAc), 23.10 (CH_3-NHAc), 37.50 (Sia-C3), 38.32 (Sia-C9), 48.18 (Sia-C5), 52.99 ($CO_2\underline{C}H_3$), 55.21, 55.23 (2×OCH_3-MPM), 61.34 (Gal'-C6), 67.32 (Gal'-C4), 67.71 (OCH_2), 67.87 (Sia-C7), 68.51 (Sia-C8), 69.27 (Gal-C6), 69.32 (Sia-C4), 69.78 (Gal-C4), 70.31, 70.37 (Gal'-C2, Gal'-C5), 71.67 (Gal'-C3), 72.14 (Sia-C6), 73.01 (Gal-C5), 73.23, 74.73 (2×$C_6H_4\underline{C}H_2$), 78.31 (Gal-C2), 80.05 (Gal-C3), 96.71 (Sia-C2), 101.02 (Gal'-C1), 103.03 (Gal-C1), 113.61, 113.70, 113.75, 126.88, 128.24, 128.42, 128.51, 128.58, 129.34, 129.37, 129.48, 129.65, 129.82, 130.08, 130.13, 131.50, 131.88, 133.02, 134.19, 159.06, 159.16 (24C, 2×C_6H_5,

2×C_6H_4), 165.59, 165.69, 167.13, 167.73, 169.60, 170.14, 170.30, 170.74, 170.94, 171.69 (10×CO);

Anal. Calcd for $C_{71}H_{90}N_2O_{28}Si$: C, 58.91; H, 6.27; N, 1.94. Found: C, 58.31; H, 6.24; N, 1.67.

2-(Trimethylsilyl)ethyl (sodium 5-acetamido-9-benzoamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-(2→3)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (**87**), (GG-290, *I-288, 290*).

To a stirred solution of **96** (23.0 mg, 15.9 μmol) in DCM (1.8 mL) were added DDQ (10.8 mg, 47.7 μmol) and H_2O (0.1 mL). The mixture was stirred at rt for 3 h, then the precipitate was filtered and washed with DCM (2×5 mL). The combined filtrates were washed with H_2O (2×5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0.5% gradient MeOH in DCM) to afford white foam (16.2mg, 84%), which was then dissolved in MeOH (1.8mL), and treated with freshly prepared 1M NaOMe/MeOH (0.2mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 3 h. The solution was neutralized by 0.5% HCl, concentrated, and the residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **87** as a white solid (10.1 mg, 88%, 74% for two steps) after a final lyophilization from H_2O.

$[\alpha]_D$: +2.2°, (*c* = 0.67, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.02 (s, 9H, $SiMe_3$), 0.96 (td, $J = J$ = 12.9, J = 5.2 Hz, $SiCH_2$-Ha), 1.05 (td, $J = J$ = 12.9, J = 5.5 Hz, $SiCH_2$-Hb), 1.80 (t, $J_{3a,3b} = J_{3a,4}$ = 12.1 Hz, Sia-H3a), 2.00 (s, 3H, CH_3-NHAc), 2.76 (dd, $J_{3a,3b}$ = 12.2, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.55-3.66 (m, 6H, Gal-H2, Gal-H5, Gal'-H2, Gal'-H5, Sia-H7, Sia-H9a), 3.67-3.78 (m, 9H, Gal-H3, Gal-H6a, Gal-H6b, Gal'-H6a, Gal'-H6b, Sia-H4, Sia-H6, Sia-H9b, OCH_2-Ha), 3.87 (t, $J_{4,5} = J_{5,6}$ = 10.2 Hz, 1H, Sia-H5), 3.94 (d, $J_{3,4}$ = 3.1 Hz, 1H, Gal'-H4), 3.99-4.07 (m, 2H, Sia-H8, OCH_2-Hb), 4.11 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 3.1 Hz, 1H, Gal'-H3), 4.14 (d, $J_{3,4}$ = 3.3 Hz, 1H, Gal-H4), 4.37 (d, $J_{1,2}$ = 8.0 Hz, 1H, Gal-H1), 4.64 (d, $J_{1,2}$ = 7.8 Hz, 1H, Gal'-H1), 7.53-7.56, 7.61-7.64, 7.78-7.80 (m, 5H, C_6H_5);

^{13}C NMR (125 MHz, D_2O): δ -2.17 (3C, $SiMe_3$), 17.90 ($SiCH_2$), 22.37 (CH_3-NHAc), 40.13 (Sia-C3), 43.06 (Sia-C9), 52.04 (Sia-C5), 61.16, 61.27 (Gal-C6, Gal'-C6), 67.77

(Gal'-C4), 68.62 (OCH_2), 68.76 (Gal-C4), 69.80 (Sia-C4), 70.06, 70.13 (3C, Gal-C2, Gal'-C2, Sia-C7), 70.72 (Sia-C8), 75.03 (2C, Gal-C5, Gal'-C5), 75.31 (Sia-C6), 76.05 (Gal'-C3), 83.05 (Gal-C3), 100.20 (Sia-C2), 102.05 (Gal-C1), 104.46 (Gal'-C1), 127.50, 129.16, 132.50, 134.06 (6C, C_6H_5), 171.64, 174.10 (2×CO).

Methyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (**98**), (GG-292, *I-292*).

To a solution of **55** (500 mg, 0.960 mmol) and benzyl alcohol (198 μL, 1.92 mmol) in CH_3CN (10 mL) was added activated powdered molecular sieves 3Å (2.00 g). The suspension was stirred at rt under argon for 5 h, then cooled to –35°C. After addition of NIS (432 mg, 1.92 mmol) and TfOH (16.9 μL, 0.192 mmol), stirring was continued for 16 h at –35°C. Then the mixture was diluted with DCM (20 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3×20 mL), and the combined filtrates were washed with 20% aq. $Na_2S_2O_3$ (2×30 mL), 5% $KHCO_3$ (2×30 mL) and H_2O (30 mL), dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/DCM/*i*PrOH 8:4:1) to afford **98** (198 mg, 35%) as white foam, the NMR data of which were in accordance with Lit.[178]

Methyl (benzyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (**99**), (GG-293, *I-293*).

To a solution of **98** (112 mg, 0.193 mmol) in MeOH (3.0 mL) was added freshly prepared 1M NaOMe/MeOH (0.3 mL). The mixture was stirred at rt for 2h under argon, then neutralized with Amberlite IRC 50 ion-exchange resin and filtered through a pad of Celite. The Celite was washed with MeOH (3×5 mL) and the combined filtrates were evaporated under reduced pressure. The residue was purified by silica gel chromatography (2% gradient MeOH in DCM) to afford **99** (61.5 mg, 78%) as white foam, the NMR data of which were in accordance with Lit.[178]

Methyl (benzyl 5-acetamido-8,9-*O*-benzylidene-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (**100**), (GG-294, *I-295*).

To a stirred solution of **99** (36.0 mg, 87.1 μmol) and α,α-dimethoxytoluene (26.0 μL, 174 μmol) in CH_3CN (2 mL) at 0°C, $TsOH \cdot H_2O$ (1.30 mg, 6.70 μmol) was added.

The mixture was stirred at rt for 1.5 h and then quenched by adding a few drops of Et_3N. After concentration under reduced pressure, the residue (~50 mg) was used directly in the next step. The ^1H NMR data of **100** showed a 1:1 mixture of *exo*/*endo* isomers, which were in accordance with Lit.[161]

Methyl (benzyl 5-acetamido-9-*O*-benzyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (**101**), (GG-296, *I-296*).

100 (87.1 μmol), $BH_3{\cdot}NMe_3$ (25.4 mg, 0.348 mmol) and $AlCl_3$ were dissolved in THF (2.0 mL) under argon. After 5 min, H_2O (2.60 μL, 0.131 mmol) was added and the mixture was stirred at rt for 4 h. Then the reaction was quenched by adding H_2O (1 mL) and 0.1 N HCl (1 mL). The solution was extracted with DCM (10 mL) and washed by 5% $NaHCO_3$ (2×5 mL) followed by 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 (1% gradient MeOH in DCM) to afford **101** (34.8 mg, 70% **99→101**) as white foam.

$[\alpha]_D$ +4.8°, (*c* = 0.51, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 1.80 (t, $J_{3a,3b} = J_{3a,4} =$ 12.5 Hz, 1H, Sia-H3a), 2.00 (s, 3H, CH_3-NHAc), 2.74 (dd, $J_{3a,3b} = 12.8$, $J_{3b,4} = 4.6$ Hz, 1H, Sia-H3b), 3.60 (dd, $J_{6,7} = 1.5$, $J_{7,8} = 8.9$ Hz, 1H, Sia-H7), 3.63-3.69 (m, 3H, Sia-H4, Sia-H6, Sia-H9a), 3.76 (s, 3H, CO_2CH_3), 3.78-3.83 (m, 2H, Sia-H5, Sia-H9b), 4.02 (ddd, $J_{7,8} = 8.6$, $J_{8,9} = 2.3$, 5.8 Hz, 1H, Sia-H8), 4.50, 4.80 (A,B of AB, J = 11.7 Hz, 2H, PhC$\underline{H}_2$), 4.57, 4.59 (A, B of AB, 2H, PhC$\underline{H}_2$), 7.24-7.37 (m, 10H, 2×C_6H_5);

^{13}C NMR (125 MHz, CD_3OD): δ 22.57 (CH_3-NHAc), 41.68 (Sia-C3), 53.21 (OCH_3), 53.76 (Sia-C5), 67.19 (Ph$\underline{C}H_2$), 68.42 (Sia-C4), 70.09 (Sia-C7), 71.34 (Sia-C8), 72.59 (Sia-C9), 74.35 (Ph$\underline{C}H_2$), 74.83 (Sia-C6), 100.05 (Sia-C2), 128.50, 128.62, 128.75, 128.82, 129.18, 129.24, 138.76 (12C, 2×C_6H_5), 170.93, 175.10 (2×CO);

Anal. Calcd for $C_{26}H_{33}NO_9$+1/2 H_2O: C, 60.93; H, 6.69; N, 2.73. Found: C, 61.14; H, 6.62; N, 2.65.

Benzyl (sodium 5-acetamido-9-*O*-benzyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**97**), (GG-297, *I-297*).

To a solution of **101** (35.0 mg, 69.5 μmol) in H_2O/dioxane (1:1, 4.0 mL) under argon was added LiOH (16.8 mg, 69.5 μmol). The mixture was stirred at rt for 1.5 h, then neutralized by 0.5% HCl and concentrated. The residue was purified by silica gel

chromatography (10% gradient MeOH in DCM), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **97** as a white solid (31.0 mg, 87%) after a final lyophilization from H_2O.

$[\alpha]_D$ -16.1°, (*c* = 0.65, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.68 (t, $J_{3a,3b} = J_{3a,4} = 12.2$ Hz, 1H, Sia-H3a), 2.03 (s, 3H, CH_3-NHAc), 2.77 (dd, $J_{3a,3b} = 12.4$, $J_{3b,4} = 4.6$ Hz, 1H, Sia-H3b), 3.60-3.64 (m, 2H, Sia-H7, Sia-H9a), 3.67-3.73 (m, 2H, Sia-H4, Sia-H6), 3.78-3.82 (m, 2H, Sia-H5, Sia-H9b), 3.86 (ddd, $J_{7,8} = 8.6$, $J_{8,9} = 2.0$, 6.3 Hz, 1H, Sia-H8), 4.49, 4.69 (A, B of AB, *J* = 11.0 Hz, 2H, PhC$\underline{H}_2$), 4.58, 4.61 (A, B of AB, *J* = 12.0 Hz, 2H, PhC$\underline{H}_2$), 7.35-7.46 (m, 10H, 2×C_6H_5);

^{13}C NMR (125 MHz, D_2O): δ 22.04 (CH_3-NHAc), 40.52 (Sia-C3), 51.89 (Sia-C5), 67.13 (Ph$\underline{C}H_2$), 68.23, 68.35 (Sia-C4, Sia-C7), 70.37 (Sia-C8), 70.81 (Sia-C9), 72.61 (Sia-C6), 73.04 (Ph$\underline{C}H_2$), 100.95 (Sia-C2), 128.19, 128.26, 128.32, 128.65, 128.68. 128.72, 137.11, 137.52 (12C, 2×C_6H_5), 173.48, 175.07 (2×CO).

Methyl (3'-phenoxybenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α and **β-D-*galacto*-2-nonulopyranosid) onate** (**109α**) and (**109β**), (GG-205-2 and GG-205-1, *I-205*).

To a solution of **55** (86.8 mg, 0.166 mmol) and 3-phenoxybenzyl alcohol **108** (43.4 µL, 0.250 mmol) in CH_3CN (6 mL) was added activated powdered molecular sieves 3Å (2.00 g). The reaction mixture was stirred at rt under argon for 5 h, then cooled to –30°C. After addition of NIS (75.0 mg, 0.332 mmol) and TfOH (3.0 µL, 33.2 µmol), stirring was continued for 16 h at –30°C. Then the mixture was diluted with DCM (20 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% aq. $Na_2S_2O_3$ (30 mL), 5% $KHCO_3$ (2×20 mL) and H_2O (20 mL), dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by silica gel chromatography (1% gradient of MeOH in DCM) to afford **109α** (66.0 mg, 58%) and **109β** (26.5 mg, 23%) as white foams.

109α: $[\alpha]_D$ +2.5°, (*c* = 1.26, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.82 (s, 3H, CH_3-NHAc), 1.95 (m, 1H, Sia-H3a), 1.96, 1.97, 2.06, 2.08 (4s, 12H, 4×CH_3-OAc), 2.57 (dd, $J_{3a,3b} = 12.8$, $J_{3b,4} = 4.6$ Hz, 1H, Sia-H3b), 3.62 (s, 3H, OCH_3), 4.01 (q, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.8$ Hz, 1H, Sia-H5), 4.03 (m, 1H, Sia-H9a), 4.07 (dd, $J_{5,6} = 10.7$, $J_{6,7} = 2.1$ Hz, 1H, Sia-H6), 4.22 (dd, $J_{8,9b} = 2.7$, $J_{9a,9b} = 12.5$ Hz, 1H, Sia-H9b), 4.32, 4.73

(A,B of AB, J = 12.2 Hz, 2H, PhC$\underline{H}_2$), 4.81 (ddd, $J_{3a,4}$ = 12.3, $J_{3b,4}$ = 4.6, $J_{4,5}$ = 10.0 Hz, 1H, Sia-H4), 5.12 (d, $J_{5,NH}$ = 10.8 Hz, 1H, Sia-NH), 5.27 (dd, $J_{6,7}$ = 2.1, $J_{7,8}$ = 8.6 Hz, 1H, Sia-H7), 5.37 (ddd, $J_{7,8}$ = 8.4, $J_{8,9a}$ = 5.5, $J_{8,9b}$ = 2.7 Hz, 1H, Sia-H8), 6.82-7.28 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ 20.75, 20.79, 20.83, 21.12 (4×CH_3-OAc), 23.19 (CH_3-NHAc), 38.69 (Sia-C3), 49.44 (Sia-C5), 52.57 ($CO_2\underline{C}H_3$), 62.33 (Sia-C9), 66.46 (Ph-$\underline{C}H_2$), 67.21 (Sia-C7), 68.37 (Sia-C4), 69.01 (Sia-C8), 72.49 (Sia-C6), 98.53 (Sia-C2), 118.11, 118.34, 118.79, 122.58, 123.18, 129.53, 129.71, 139.28, 157.14, 157.18 (12C, C_6H_4, C_6H_5), 168.33, 170.04, 170.14, 170.21, 170.63, 171.01 (6×CO);

109β: $[\alpha]_D$ –8.1°, (c = 0.82, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.81 (s, 3H, CH_3-NHAc), 1.86 (m, 1H, Sia-H3a), 1.89, 1.94, 1.95, 2.08 (4s, 12H, 4×CH_3-OAc), 2.47 (dd, $J_{3a,3b}$ = 13.0, $J_{3b,4}$ = 5.0 Hz, 1H, Sia-H3b), 3.67 (s, 3H, OCH_3), 3.90 (dd, $J_{5,6}$ = 10.5, $J_{6,7}$ = 2.2 Hz, 1H, Sia-H6), 4.03 (dd, $J_{8,9a}$ = 7.6, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9a), 4.08 (q, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.4 Hz, 1H, Sia-H5), 4.41, 4.46 (A, B of AB, J = 12.1 Hz, 2H, PhC$\underline{H}_2$), 4.74 (dd, $J_{8,9b}$ = 2.5, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9b), 5.17 (m, 1H, Sia-H8), 5.21 (m, 1H, Sia-H4), 5.26 (d, $J_{5,NH}$ = 10.2 Hz, 1H, Sia-NH), 5.32 (dd, $J_{6,7}$ = 2.2, $J_{7,8}$ = 4.3 Hz, 1H, Sia-H7), 6.85-7.30 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ 20.77, 20.85 (4C, 4×CH_3-OAc), 23.14 (CH_3-NHAc), 38.20 (Sia-C3), 48.31 (Sia-C5), 51.73 ($CO_2\underline{C}H_3$), 62.32 (Sia-C9), 65.33 (Ph$\underline{C}H_2$), 68.22 (Sia-C7), 68.85 (Sia-C4), 71.73, 71.85 (Sia-C6, Sia-C8), 98.54 (Sia-C2), 117.61, 118.06, 119.08, 121.97, 122.39, 129.79, 129.85, 138.69, 156.95, 157.48 (12C, C_6H_4, C_6H_5), 170.28, 170.63, 170.96 (6C, 6×CO).

Sodium (3'-phenoxybenzyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (**104α**), (GG-208, *I-208*).

To a solution of **109α** (66.0 mg, 98.0 μmol) in MeOH (4.5 mL) was added freshly prepared 1M NaOMe/MeOH (0.5mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type) and P2 size exclusion chromatography to afford **104α** as a white solid (30.0 mg, 60%) after a final lyophilization from H_2O.

$[\alpha]_D$ –27.3°, (*c* = 0.75, H_2O); ^1H NMR (500 MHz, D_2O) δ 1.58 (t, $J_{3a,3b} = J_{3a,4} = 12.1$ Hz, 1H, Sia-H3a), 1.94 (s, 3H, CH_3-NHAc), 2.66 (dd, $J_{3a,3b} = 12.3$, $J_{3b,4} = 4.4$ Hz, 1H, Sia-H3b), 3.50 (m, 1H, Sia-H7), 3.52 (dd, $J_{8,9a} = 5.9$, $J_{9a,9b} = 12.1$ Hz, 1H, Sia-H9a), 3.58 (m, 1H, Sia-H4), 3.62 (m, 1H, Sia-H6), 3.66 (m, 1H, Sia-H8), 3.70-3.80 (m, 2H, Sia-H5, Sia-H9b), 4.39, 4.62 (A, B of AB, *J* = 11.2 Hz, 2H, $C_6H_4C\underline{H}_2$), 6.91-6.92, 6.97-6.98, 7.09-7.16, 7.26-7.34 (m, 9H, C_6H_4, C_6H_5);
^{13}C NMR (125 MHz, $CDCl_3$): δ 22.37 (CH_3-NHAc), 40.79 (Sia-C3), 52.22 (Sia-C5), 62.84 (Sia-C9), 66.88 ($C_6H_4\underline{C}H_2$), 68.48 (Sia-C7), 68.64 (Sia-C4), 71.99 (Sia-C8), 73.06 (Sia-C6), 101.26 (Sia-C2), 118.83, 119.07, 119.29, 124.11, 124.25, 130.46, 130.56, 139.75, 157.00, 157.23 (12C, C_6H_4, C_6H_5), 173.81, 175.44 (2×CO).

Sodium (3'-phenoxybenzyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid) onate (**104β**), (GG-207, *I-207*).

To a solution of **109β** (25.0 mg, 37.1 μmol) in MeOH (4.5 mL) was added freshly prepared 1M NaOMe/MeOH (0.5mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **104β** as a white solid (12.0 mg, 63%) after a final lyophilization from H_2O.

$[\alpha]_D$ +7.8°, (*c* = 0.62, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.39 (dd, $J_{3a,3b} = 13.0$, $J_{3a,4} = 11.6$ Hz, 1H, Sia-H3a), 1.75 (s, 3H, CH_3-NHAc), 2.10 (dd, $J_{3a,3b} = 13.1$, $J_{3b,4} = 4.9$ Hz, 1H, Sia-H3b), 3.25 (d, $J_{5,6} = 9.6$ Hz, 1H, Sia-H6), 3.38 (dd, $J_{8,9a} = 5.5$, $J_{9a,9b} = 12.0$ Hz, 1H, Sia-H9a), 3.53 (dd, $J_{8,9a} = 2.7$, $J_{9a,9b} = 12.0$ Hz, 1H, Sia-H9b), 3.59-3.63 (m, 3H, Sia-H5, Sia-H7, Sia-H8), 3.75 (m, 1H, Sia-H4), 3.98, 4.28 (A, B of AB, *J* = 10.4 Hz, 2H, $C_6H_4C\underline{H}_2$), 6.73-6.75, 6.79-6.81, 6.86, 6.91-6.97, 7.11-7.16 (m, 9H, C_6H_4, C_6H_5);
^{13}C NMR (125 MHz, D_2O): δ 22.27 (CH_3-NHAc), 40.84 (Sia-C3), 52.69 (Sia-C5), 64.14 (Sia-C9), 65.11 ($C_6H_4\underline{C}H_2$), 67.68 (Sia-C4), 68.88 (Sia-C7), 70.61 (Sia-C6), 71.02 (Sia-C8), 100.78 (Sia-C2), 119.12, 119.54, 119.63, 124.56, 124.61, 130.83, 130.93, 140.10, 157.40, 157.60 (12C, C_6H_4, C_6H_5), 175.81 (2C, 2×CO).

Methyl (4'-biphenylmethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α and **β-D-*galacto*-2-nonulopyranosid) onate** (**111α**) and (**111β**), (GG-198-2 and GG-198-1, *I-198*).

To a solution of **55** (94.4 mg, 0.181 mmol) and 4-biphenyl-methanol **110** (50.0 mg, 0.271 mmol) in CH_3CN (6 mL) was added activated powdered molecular sieves 3Å (2.00 g). The reaction mixture was stirred at rt under argon for 5 h, then cooled to –30°C. After addition of NIS (81.4 mg, 0.362 mmol) and TMSOTf (6.5 μL, 36.2 μmol), stirring was continued for 16 h at –30°C. Then the mixture was diluted with DCM (20 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% aq. $Na_2S_2O_3$ (30 mL), 5% $KHCO_3$ (2×20 mL) and H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (toluene/AcOEt 2:1) to afford **111α** (75.0 mg, 63%) and **111β** (24.5 mg, 21%) as white foams.

111α: $[\alpha]_D$ +5.5°, (*c* = 0.87, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82, 1.96, 1.98, 2.08, 2.11 (5s, 15H, CH_3-NHAc, 4×CH_3-OAc), 1.99 (m, 1H, Sia-H3a), 2.61 (dd, $J_{3a,3b}$ = 12.8, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.63 (s, 3H, OCH_3), 4.01-4.07 (m, 2H, Sia-H5, Sia-H9a), 4.09 (dd, $J_{5,6}$ = 12.7, $J_{6,7}$ = 2.1 Hz, 1H, Sia-H6), 4.28 (dd, $J_{8,9b}$ = 2.7, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9b), 4.40, 4.79 (A of AB, *J* = 12.0 Hz, 1H, $C_6H_4C\underline{H}_2$), 4.82 (ddd, $J_{3a,4}$ = 12.3, $J_{3b,4}$ = 4.6, $J_{4,5}$ = 9.9 Hz, 1H, Sia-H4), 5.20 (d, $J_{5,NH}$ = 9.7 Hz, 1H, Sia-NH), 5.29 (dd, $J_{6,7}$ = 2.0, $J_{7,8}$ = 8.5 Hz, 1H, Sia-H7), 5.37 (ddd, $J_{7,8}$ = 8.4, $J_{8,9a}$ = 5.6, $J_{8,9b}$ = 2.7 Hz, 1H, Sia-H8), 7.26-7.29, 7.33-7.38, 7.49-7.52 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ 20.76, 20.82, 20.84, 21.14, 23.17 (CH_3-NHAc, 4×CH_3-OAc), 38.11 (Sia-C3), 49.42 (Sia-C5), 52.65 ($CO_2\underline{C}H_3$), 62.39 (Sia-C9), 66.59 ($C_6H_4\underline{C}H_2$), 67.27 (Sia-C7), 68.51 (Sia-C8), 68.07 (Sia-C4), 72.51 (Sia-C6), 98.53 (Sia-C2), 126.99, 127.03, 127.27, 128.32, 128.74, 129.00, 136.12, 140.66, 140.84 (12C, C_6H_4, C_6H_5), 168.39, 170.12, 170.15, 170.25, 170.66, 171.02 (6×CO);

111β: $[\alpha]_D$ –15.5°, (*c* = 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.88 (dd, $J_{3a,3b}$ = 13.0, $J_{3a,4}$ = 11.6 Hz, 1H, Sia-H3a), 1.81, 1.91, 1.95, 1.98, 2.09 (5s, 15H, CH_3-NHAc, 4×CH_3-OAc), 2.51 (dd, $J_{3a,3b}$ = 13.0, $J_{3b,4}$ = 5.0 Hz, 1H, Sia-H3b), 3.68 (s, 3H, OCH_3), 3.96 (dd, $J_{5,6}$ = 10.5, $J_{6,7}$ = 2.2 Hz, 1H, Sia-H6), 4.06 (dd, $J_{8,9a}$ = 7.8, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9a), 4.08 (q, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.3 Hz, 1H, Sia-H5), 4.48, 4.53 (A, B of AB, *J* = 11.9 Hz, 2H, $C_6H_4C\underline{H}_2$), 4.80 (dd, $J_{8,9b}$ = 2.5, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9b),

5.21-5.28 (m, 2H, Sia-H4, Sia-H8), 5.32 (d, $J_{5,NH}$ = 10.2 Hz, 1H, Sia-NH), 5.35 (dd, $J_{6,7}$ = 2.2, $J_{7,8}$ = 4.1 Hz, 1H, Sia-H7), 7.27-7.54 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ 20.79, 20.82, 20.87, 20.92, 23.13 (4×CH_3-OAc, CH_3-NHAc), 37.42 (Sia-C3), 49.38 (Sia-C5), 52.73 ($CO_2\underline{C}H_3$), 60.40 (Sia-C9), 65.55 ($C_6H_4\underline{C}H_2$), 68.39 (Sia-C7), 68.99 (Sia-C8), 71.88 (2C, Sia-C4, Sia-C6), 98.52 (Sia-C2), 127.07, 127.25, 127.40, 127.98, 128.79, 135.57, 140.68, 140.92 (12C, C_6H_4, C_6H_5), 167.38, 170.15, 170.30, 170.64, 170.75, 171.02 (6×CO).

Sodium (4'-biphenylmehtyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (**106α**), (GG-200, *I-200*).

To a solution of **111α** (51.3 mg, 78.0 μmol) in MeOH (4.5 mL) was added freshly prepared 1M NaOMe/MeOH (0.5mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **106α** as a white solid (23.0 mg, 60%) after a final lyophilization from H_2O.

$[\alpha]_D$ –22.4°, (*c* = 0.74, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.42 (t, $J_{3a,3b} = J_{3a,4}$ = 12.1 Hz, 1H, Sia-H3a), 1.74 (s, 3H, CH_3-NHAc), 2.50 (dd, $J_{3a,3b}$ = 12.4, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.30 (dd, $J_{6,7}$ = 1.8, $J_{7,8}$ = 9.4 Hz, 1H, Sia-H7), 3.32 (dd, $J_{8,9a}$ = 5.8, $J_{9a,9b}$ = 12.1 Hz, 1H, Sia-H9a), 3.58 (m, 3H, Sia-H4, Sia-H6, Sia-H8), 3.52 (dd, $J_{8,9a}$ = 2.3, $J_{9a,9b}$ = 12.0 Hz, 1H, Sia-H9b), 3.54 (t, $J_{4,5} = J_{5,6}$ = 10.1 Hz, 1H, Sia-H5), 4.27, 4.47 (A, B of AB, *J* = 11.3 Hz, 2H, $C_6H_4C\underline{H}_2$), 7.11-7.14, 7.17-7.25, 7.36-7.40 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ 22.37 (CH_3-NHAc), 40.91 (Sia-C3), 52.25 (Sia-C5), 62.79 (Sia-C9), 67.22 ($C_6H_4\underline{C}H_2$), 68.45 (Sia-C7), 68.67 (Sia-C4), 71.99 (Sia-C8), 73.09 (Sia-C6), 101.46 (Sia-C2), 127.30, 127.38, 128.13, 129.49, 129.66, 136.91, 140.55, 140.64 (12C, C_6H_4, C_6H_5), 174.00, 175.05 (2×CO).

Sodium (4'-biphenylmethyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid) onate (**106β**), (GG-202, *I-202*).

To a solution of **111β** (24.5 mg, 37.3 μmol) in MeOH (4.5 mL) was added freshly prepared 1M NaOMe/MeOH (0.5mL). The mixture was stirred at rt for 5 h under

argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **106β** as a white solid (10.5 mg, 57%) after a final lyophilization from H_2O.

$[\alpha]_D$ +21.2°, (*c* = 0.70, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.53 (t, $J_{3a,3b} = J_{3a,4} = 12.3$ Hz, 1H, Sia-H3a), 1.87 (s, 3H, CH_3-NHAc), 2.24 (dd, $J_{3a,3b} = 13.2$, $J_{3b,4} = 4.9$ Hz, 1H, Sia-H3b), 3.41 (d, $J_{7,8} = 9.7$ Hz, 1H, Sia-H7), 3.51 (dd, $J_{8,9a} = 5.6$, $J_{9a,9b} = 12.0$ Hz, 1H, Sia-H9a), 3.69 (dd, $J_{8,9a} = 2.3$, $J_{9a,9b} = 12.0$ Hz, 1H, Sia-H9b), 3.73-3.80 (m, 2H, Sia-H5, Sia-H8), 3.83 (d, $J_{5,6} = 10.5$ Hz, 1H, Sia-H6), 3.90 (m, 1H, Sia-H4), 4.15, 4.47 (A,B of AB, *J* = 10.2 Hz, 2H, $C_6H_4C\underline{H}_2$), 7.24-7.27, 7.33-7.39, 7.52-7.53 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ 22.48 (CH_3-NHAc), 40.31 (Sia-C3), 52.43 (Sia-C5), 63.96 (Sia-C9), 64.97 ($C_6H_4\underline{C}H_2$), 67.39 (Sia-C4), 68.63 (Sia-C7), 70.36 (Sia-C8), 70.68 (Sia-C6), 100.50 (Sia-C2), 127.33, 127.46, 128.17, 129.52, 129.79, 136.88, 140.56, 140.71 (12C, C_6H_4, C_6H_5), 175.15, 175.62 (2×CO).

Methyl (4'-phenoxyphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside) onate (**113α**), (GG-214, *I-214*).

To a solution of **8** (100 mg, 0.196 mmol) in $CHCl_3$ (5 mL) at rt was added 4-phenoxyphenol **112** (183 mg, 0.981 mmol) dissolved in 0.2M aq. NaOH (5 mL), followed by benzyltriethylammonium chloride (98.0 mg, 0.431 mmol). The reaction mixture was stirred vigorously at 100°C for 2 h, then diluted with DCM (20 mL), washed with 0.1M NaOH (2×10 mL), H_2O (2×10 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (0.25% gradient of MeOH in DCM) to afford **113α** (78.0 mg, 60%) as white foam.

$[\alpha]_D$ -2.2° (*c* = 1.12, $CHCl_3$); ^{1}H NMR (500 Hz, $CDCl_3$): δ 1.83 (s, 3H, CH_3-NHAc), 1.95, 1.97, 2.05, 2.06 (4s, 12H, 4×CH_3-OAc), 2.12 (t, $J_{3a,3b} = J_{3a,4} = 12.6$ Hz, 1H, Sia-H3a), 2.64 (dd, $J_{3a,3b} = 12.9$, $J_{3b,4} = 4.7$ Hz, 1H, Sia-H3b), 3.62 (s, 3H, OCH_3), 4.02 (q, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.4$ Hz, 1H, Sia-H5), 4.09 (dd, $J_{8,9a} = 4.7$, $J_{9a,9b} = 12.2$ Hz, 1H, Sia-H9a), 4.24-4.27 (m, 2H, Sia-H6, Sia-H9b), 4.88 (ddd, $J_{3b,4} = 4.6$, $J_{3a,4} = 12.2$, $J_{4,5} = 10.5$

Hz, 1H, Sia-H4), 5.25 (d, $J_{5,NH}$ = 10.1 Hz, 1H, Sia-NH), 5.27-5.31 (m, 2H, Sia-H7, Sia-H8), 6.85-6.87, 6.89-6.92, 6.96-7.02, 7.23-7.26 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, $CDCl_3$): δ 20.72, 20.82, 20.94 (4C, 4×CH_3-OAc), 23.17 (CH_3-NHAc), 37.74 (Sia-C3), 49.31 (Sia-C5), 52.88 (OCH_3), 62.04 (Sia-C9), 67.40 (Sia-C7), 68.86 (Sia-C4), 69.31 (Sia-C8), 73.29 (Sia-C6), 100.33 (Sia-C2), 118.28, 119.85, 122.01, 122.95 129.66, 149.35, 153.54, 157.80 (12C, C_6H_4, C_6H_5), 167.77, 169.95, 169.98, 170.25, 170.59, 170.92 (6×CO);

HRMS (FAB): Calcd for $C_{32}H_{37}NO_{14}$ $(M+Na)^+$: 682.2214; Found m/z 682.2212.

Sodium (4'-phenoxyphenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate (**105α**), (GG-215, *I-215*).

To a solution of **113α** (55.9 mg, 84.7 μmol) in MeOH (4.5 mL) was added freshly prepared 1M NaOMe/MeOH (0.5mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **105α** as a white solid (34.1 mg, 81%) after a final lyophilization from H_2O.

$[\alpha]_D$ +29.4° (*c* = 0.85, H_2O); ^{1}H NMR (500 Hz, D_2O): δ 1.73 (t, $J_{3a,3b} = J_{3a,4} = 11.9$ Hz, 1H, Sia-H3a), 1.85 (s, 3H, CH_3-NHAc), 2.73 (dd, $J_{3a,3b} = 12.2$, $J_{3b,4} = 3.6$ Hz, 1H, Sia-H3b), 3.41-3.45 (m, 2H, Sia-H7, Sia-H9a), 3.56 (m, 1H, Sia-H4), 3.61-3.74 (m, 4H, Sia-H5, Sia-H6, Sia-H8, Sia-H9b), 6.78-6.80, 6.84-6.85, 6.97-6.99, 7.19-7.22 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, D_2O): δ 22.16 (CH_3-NHAc), 40.80 (Sia-C3), 51.67 (Sia-C5), 62.17 (Sia-C9), 68.31 (2C, Sia-C4, Sia-C7), 72.07 (Sia-C8), 73.35 (Sia-C6), 103.00 (Sia-C2), 118.51, 119.79, 123.51, 123.71, 130.17, 149.67, 153.31, 157.24 (12C, C_6H_4, C_6H_5), 172.64, 175.22 (2×CO).

Methyl 4-(4',4',5',5'-tetramethyl-1',2',3'-dioxaborolan-2'-yl)-phenylacetate (**115**), (GG-2-006, *II-006, 008*).

To a solution of 4-bromophenylacetic acid **116** (700 mg, 3.25 mmol) in MeOH (20 mL) at rt was added CAN (2.68 g, 4.88 mmol). The mixture was stirred for 16 h, and then evaporated under reduced pressure. The residue was diluted by DCM (50mL),

washed with H_2O (2×30 mL), and dried over Na_2SO_4. After filtration and concentration, methyl 4-bromophenylacetate (660 mg, 90%) was obtained as colorless oil. Without purification, methyl 4-bromophenylacetate (150 mg, 655 μmol) was added to a MW tube together with bis(pinacolato)diboron (200 mg, 786 μmol), dry KOAc (193 mg, 1.96 mmol), $PdCl_2$(dppf) (16.0 mg, 19.7 μmol) and dppf (10.9 mg, 19.7 μmol), and dried under HV for 15 min. Then dioxane (5 mL) was added under argon, and the mixture was sonicated for 15 min and irradiated at 120°C for 1.5 h. The reaction was then evaporated under reduced pressure, and the residue was diluted with DCM (50 mL), washed with H_2O (2×50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (1% gradient of AcOEt in toluene) to afford **115** (91.6 mg, 50%) as white foam.

^{1}H NMR (500 Hz, $CDCl_3$): δ 1.33 (s, 12H, 4×CH_3), 3.64 (s, 2H, C$\underline{H}_2$Ph), 3.68 (s, 3H, OCH_3), 7.29, 7.77 (AA', BB' of AA'BB', J=8.0 Hz, 4H, C_6H_4).

Methyl 4-[3-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-phenyl]-phenylacetate (**117**) and **sodium 4-[3-*O*-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate)-phenyl]-phenylacetate** (**107**), (GG-2-013-3 and GG-2-013-10, *II-013*).

114[196] (149 mg, 0.230 mmol), BHT (35.5 mg, 0.160 mmol), dry K_3PO_4 (146.5 mg, 0.690 mmol), $PdCl_2$(dppf) (5.60 mg, 6.90 μmol) and dppf (3.80 mg, 6.90 μmol) were added to a MW tube, and dried under HV at 60°C for 15 min. Then **115** (70.0 mg, 0.254 mmol) and dioxane (5 mL) were added under argon. The mixture was sonicated for 20 min, and irradiated at 170°C for 2.25 h. The reaction was evaporated under reduced pressure, and the residue was diluted with DCM (50 mL) and washed with H_2O (2×50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/AcOEt 3:1 to 1:6) to afford **117** (12.5 mg, 8%) as white foam. The H_2O layer was concentrated under reduce pressure to give a brown solid, which was stirred with cold MeOH (10 mL). After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (MeOH/H_2O 10:1), RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and

P2 size exclusion chromatography to afford **107** as a white solid (4.00 mg, 3%) after a final lyophilization from H_2O.

117: $[\alpha]_D$ +11.4° (*c* = 0.67, $CHCl_3$); ^1H NMR (500 Hz, $CDCl_3$): δ 1.90 (s, 3H, CH_3-NHAc), 2.01, 2.02, 2.03, 2.11 (4s, 12H, 4×CH_3-OAc), 2.20 (t, $J_{3a,3b} = J_{3a,4} = 12.6$ Hz, 1H, Sia-H3a), 2.69 (dd, $J_{3a,3b} = 13.0$, $J_{3b,4} = 4.7$ Hz, 1H, Sia-H3b), 3.66 (s, 2H, $C_6H_4C\underline{H}_2$), 3.70, 3.71 (2s, 6H, 2×OCH_3), 4.06 (q, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.4$ Hz, 1H, Sia-H5), 4.18 (dd, $J_{8,9a} = 5.5$, $J_{9a,9b} = 12.5$ Hz, 1H, Sia-H9a), 4.33-4.36 (m, 2H, Sia-H6, Sia-H9b), 4.99 (ddd, $J_{3b,4} = 4.7$, $J_{3a,4} = 12.1$, $J_{4,5} = 10.4$ Hz, 1H, Sia-H4), 5.24 (d, $J_{5,NH} = 10.1$ Hz, 1H, Sia-NH), 5.34 (dd, $J_{6,7} = 1.9$, $J_{7,8} = 7.7$ Hz, 1H, Sia-H7), 5.40 (m, 1H, Sia-H8), 7.08-7.10, 7.29-7.30, 7.33-7.35, 7.54-7.55 (m, 8H, C_6H_4, C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ 20.60, 20.77, 20.88, 20.99 (4×CH_3-OAc), 23.24 (CH_3-NHAc), 37.56 (Sia-C3), 40.83 ($C_6H_4\underline{C}H_2$), 49.46 (Sia-C5), 52.13 (OCH_3), 62.01 (Sia-C9), 67.47 (Sia-C7), 68.89 (Sia-C4), 69.52 (Sia-C8), 73.39 (Sia-C6), 100.12 (Sia-C2), 119.23, 119.59, 122.98, 127.29, 129.72, 129.74, 133.35, 139.28, 142.03, 153.81 (12C, C_6H_4, C_6H_4), 160.08. 170.06, 170.08, 170.26, 170.62, 170.97 (6× CO);

107: $[\alpha]_D$ +12.5° (*c* = 0.26, H_2O); ^1H NMR (500 Hz, D_2O): δ 1.96 (t, $J_{3a,3b} = J_{3a,4} = 12.2$ Hz, 1H, Sia-H3a), 2.04 (s, 3H, CH_3-NHAc), 2.86 (dd, $J_{3a,3b} = 12.5$, $J_{3b,4} = 4.7$ Hz, 1H, Sia-H3b), 3.41-3.45 (m, 2H, Sia-H7, Sia-H9a), 3.57 (s, 2H, $C_6H_4C\underline{H}_2$), 3.60-3.65 (m, 2H, Sia-H7, Sia-H9a), 3.75 (ddd, $J_{3b,4} = 4.7$, $J_{3a,4} = 12.0$, $J_{4,5} = 9.2$ Hz, 1H, Sia-H4), 3.80 (dd, $J_{8,9b} = 2.4$, $J_{9a,9b} = 12.1$ Hz, 1H, Sia-H9b), 3.87-3.94 (m, 3H, Sia-H5, Sia-H6, Sia-H8), 7.12-7.14, 7.38, 7.40-7.50, 7.63 (m, 8H, C_6H_4, C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ 22.31 (CH_3-NHAc), 41.29 (Sia-C3), 44.41 ($C_6H_4\underline{C}H_2$), 52.05 (Sia-C5), 62.94 (Sia-C9), 68.38 (2C, Sia-C4, Sia-C7), 72.31 (Sia-C8), 73.67 (Sia-C6), 103.12 (Sia-C2), 120.13, 120.52, 123.20, 127.29, 130.06, 130.16, 138.29, 141.86, 153.33, 154.59 (12C, C_6H_4, C_6H_4).

Methyl (4'-phenoxyphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α and **β-D-*galacto*-2-nonulopyranosid)onate** (**113α**) and (**113β**), (GG-214 and GG-209-1, *I-268*).

DEAD (47.5 μL, 0.3 mmol) was added to a stirred solution of **118** (100 mg, 0.204 mmol), Ph_3P (80.0 mg, 0.306 mmol) and 4-phenoxyphenol **112** (75.8 mg, 0.408 mmol) in dry CH_3CN (6 mL) at 0°C under argon. The reaction mixture was stirred at

0°C for 3 h, diluted with DCM (15 mL) and filtered through a pad of Celite. The Celite was washed with DCM (3×5 mL), and the combined filtrates were concentrated under reduced pressure, dried at HV. A catalytic amount of $RuCl_3 \cdot H_2O$ was added to a vigorously stirred biphasic solution of the residue in $NaIO_4$ (~100 mg), CCl_4 (0.8 mL), CH_3CN (0.8 mL) and H_2O (1.2 mL). After 5 min at rt the thick, yellowish-green mixture was diluted with DCM (25 mL), and washed with H_2O (2×10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (0.25% gradient MeOH in DCM) to afford **113** (101mg, 75%, α:β =76:24) as white foam. The diastereomeric mixture of **113** could be separated by silica gel chromatography (5% gradient EtOAc in toulene), yielding **113α** (60.0 mg, 45%) and **113β** (16.0 mg, 12%) as white foams. The data of **113α** were in accordance with the data obtained above.

113β: $[\alpha]_D$ -31.3° (*c* = 0.74, $CHCl_3$); ^{1}H NMR (500 Hz, $CDCl_3$): δ 1.72, 1.73, 1.83, 1.91, 2.01 (5s, 15H, 4×CH_3-OAc, CH_3-NHAc), 1.85 (m, 1H, Sia-H3a), 2.52 (dd, $J_{3a,3b}$ = 12.9, $J_{3b,4}$ = 4.9 Hz, 1H, Sia-H3b), 3.60 (s, 3H, OCH_3), 3.96 (dd, $J_{5,6}$ = 10.6, $J_{6,7}$ = 2.3 Hz, 1H, Sia-H6), 4.00 (dd, $J_{8,9a}$ = 7.1, $J_{9a,9b}$ = 12.6 Hz, 1H, Sia-H9a), 4.08 (dd, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.4 Hz, 1H, Sia-H5), 4.55 (dd, $J_{8,9b}$ = 2.2, $J_{9a,9b}$ = 12.5 Hz, 1H, Sia-H9b), 4.75 (ddd, $J_{7,8}$ = 3.9, $J_{8,9a}$ = 7.1, $J_{8,9b}$ = 2.2 Hz, 1H, Sia-H8), 5.23(dd, $J_{6,7}$ = 2.3, $J_{7,8}$ = 3.9 Hz, 1H, Sia-H7), 5.29(d, $J_{5,NH}$ = 10.2 Hz, 1H, Sia-NH), 5.32 (td, $J_{3a,4}$ = $J_{4,5}$ = 11.1, $J_{3b,4}$ = 4.9, 1H, Sia-H4), 6.74-6.76, 6.80-6.91, 6.91-6.94, 7.15-7.18 (m, 9H, C_6H_4, C_6H_5);
^{13}C NMR (125 MHz, $CDCl_3$): δ 19.69, 19.75, 19.81. 19.88 (4×CH_3-OAc), 22.13 (CH_3-NHAc), 37.34 (Sia-C3), 48.12 (Sia-C5), 52.14 (OCH_3), 61.17 (Sia-C9), 67.17 (Sia-C7), 67.51 (Sia-C4), 71.21 (Sia-C8), 71.54 (Sia-C6), 98.18 (Sia-C2), 117.21, 117.29, 119.29. 122.01, 128.79, 148.44, 151.38, 156.56 (12C, C_6H_4, C_6H_5), 166.29, 169.11. 169.28, 169.47, 169.60, 170.03 (6×CO);
HRMS (FAB): Calcd for $C_{32}H_{37}NO_{14}$ $(M+Na)^+$: 682.2214; Found m/z 682.2225.

Sodium (4'-phenoxyphenyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid) onate (**105β**), (GG-210, *I-210*).

To a solution of **113β** (14.8 mg, 22.4 μmol) in MeOH (4.5 mL) was added freshly prepared 1M NaOMe/MeOH (0.5mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The

solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **105β** as a white solid (10.6 mg, 95%) after a final lyophilization from H_2O.

$[\alpha]_D$ –45.5° (*c* = 0.73, H_2O); ^{1}H NMR (500 Hz, D_2O): δ 1.65 (dd, $J_{3a,3b}$ = 13.0, $J_{3a,4}$ = 11.5 Hz, 1H, Sia-H3a), 1.89 (s, 3H, CH_3-NHAc), 2.41 (dd, $J_{3a,3b}$ = 13.1, $J_{3b,4}$ = 4.9 Hz, 1H, Sia-H3b), 3.31 (d, $J_{7,8}$ = 9.3 Hz, 1H, Sia-H7), 3.44 (dd, $J_{8,9a}$ = 5.5, $J_{9a,9b}$ = 11.8 Hz, 1H, Sia-H9a), 3.55 (m, 1H, Sia-H8), 3.59 (dd, $J_{8,9a}$ = 2.6, $J_{9a,9b}$ = 11.8 Hz, 1H, Sia-H9b), 3.63 (d, $J_{5,6}$ = 10.5 Hz, 1H, Sia-H6), 3.85 (t, $J_{4,5}$ = $J_{5,6}$ = 10.3 Hz, 1H, Sia-H5), 4.12 (ddd, $J_{3b,4}$ = 4.9, $J_{3a,4}$ = 11.2, $J_{4,5}$ = 10.3 Hz, 1H, Sia-H4), 6.85, 6.89, 6.92-6.94, 6.99-7.02, 7.23-7.26 (m, 9H, C_6H_4, C_6H_5);

^{13}C NMR (125 MHz, D_2O): δ 22.47 (CH_3-NHAc), 41.00 (Sia-C3), 52.22 (Sia-C5), 63.48 (Sia-C9), 67.17 (Sia-C4), 68.71 (Sia-C7), 72.61 (Sia-C8), 71.47 (Sia-C6), 100.85 (Sia-C2), 118.30, 118.35, 120.66, 122.93, 123.68, 125.72, 126.19, 130.37, 150.97, 151.11, 157.91 (12C, C_6H_4, C_6H_5), 175.12, 175.18 (2×CO).

Methyl (3'-phenylacetylene 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside)onate (**124**), (GG-300, *I-300*).

To a solution of **8** (295 mg, 0.579 mmol) in $CHCl_3$ (10 mL) was added 3-hydroxyphenylacetylene **123** (0.300 mL, 2.89 mmol) dissolved in 0.2M aq. NaOH (10 mL) at rt, followed by benzyltriethylammonium chloride (290 mg, 1.27 mmol). The reaction mixture was stirred vigorously at 100°C for 2 h, then diluted with DCM (30 mL), washed with 0.1M NaOH (2×20 mL), H_2O (2×20 mL) and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (0.5% gradient of MeOH in DCM) to afford **124** (205 mg, 60%) as white foam.

$[\alpha]_D$ +13.1° (*c* = 0.90, $CHCl_3$); ^{1}H NMR (500 Hz, $CDCl_3$): δ 1.91 (s, 3H, CH_3-NHAc), 2.04, 2.05, 2.13, 2.14 (4s, 12H, 4×CH_3-OAc), 2.21 (m, 1H, Sia-H3a), 2.64 (dd, $J_{3a,3b}$ = 13.0, $J_{3b,4}$ = 4.7 Hz, 1H, Sia-H3b), 3.07 (s, 1H, C≡C<u>H</u>), 3.70 (s, 3H, OCH_3), 4.08 (q, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.4 Hz, 1H, Sia-H5), 4.19 (dd, $J_{8,9a}$ = 4.9, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9a), 4.32-4.37 (m, 2H, Sia-H6, Sia-H9b), 4.97 (ddd, $J_{3b,4}$ = 4.7, $J_{3a,4}$ = 11.9, $J_{4,5}$ = 10.5 Hz, 1H, Sia-H4), 5.34-5.38 (m, 2H, Sia-H7, Sia-H8), 5.40 (d, $J_{5,NH}$ = 10.2 Hz, 1H, Sia-NH), 7.11-7.28 (m, 4H, C_6H_4);

^{13}C NMR (125 MHz, $CDCl_3$): δ 20.56, 20.60, 20.66, 20.79 (4×CH_3-OAc), 23.01 (CH_3-NHAc), 37.39 (Sia-C3), 49.16 (Sia-C5), 52.86 (OCH_3), 61.92 (Sia-C9), 67.19 (Sia-C7), 68.58 (Sia-C4), 69.18 (Sia-C8), 73.22 (Sia-C6), 82.79 (C≡CH), 99.89 (Sia-C2), 120.96, 124.06, 127.91, 129.24, (4C, C_6H_4-CH), 122.93, 153.10 (3C, C_6H_4-C, C≡CH), 167.66, 169.86, 169.90, 170.08, 170.44, 170.74 (6×CO);

Anal. Calcd for $C_{28}H_{33}NO_{13}$: C, 56.85; H, 5.62; N, 2.37. Found: C, 56.24; H, 5.52; N, 2.37.

Sodium (3'-phenylacetylene 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside) onate (**131**), (GG-2-027, *II-027).*

To a solution of **124** (45.0 mg, 76.1 μmol) in MeOH (2.0 mL) was added freshly prepared 1M NaOMe/MeOH (0.2mL). The mixture was stirred at rt for 3 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 2 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **131** as a white solid (27.5 mg, 84%) after a final lyophilization from H_2O.

$[\alpha]_D$ +24.3° (*c* = 0.91, H_2O); ^{1}H NMR (500 Hz, D_2O): δ 1.92 (t, $J_{3a,3b} = J_{3a,4} = 12.2$ Hz, 1H, Sia-H3a), 2.03 (s, 3H, CH_3-NHAc), 2.88 (dd, $J_{3a,3b} = 12.5$, $J_{3a,4} = 4.7$ Hz, 1H, Sia-H3b), 3.49 (s, 1H, C≡CH), 3.60 (dd, $J_{6,7} = 1.5$, $J_{7,8} = 9.2$ Hz, 1H, Sia-H7), 3.63 (dd, $J_{8,9a} = 6.3$, $J_{9a,9b} = 12.2$ Hz, 1H, Sia-H9a), 3.74 (ddd, $J_{3a,4} = 11.8$, $J_{3b,4} = 4.7$, $J_{4,5} = 9.6$ Hz, 1H, Sia-H4), 3.84-3.93 (s, 4H, Sia-H5, Sia-H6, Sia-H8, Sia-H9b), 7.18-7.22, 7.31-7.32 (m, 4H, C_6H_4);

^{13}C NMR (125 MHz, D_2O): δ 22.39 (CH_3-NHAc), 41.11 (Sia-C3), 52.09 (Sia-C5), 62.95 (Sia-C9), 68.45, 68.51 (Sia-C4, Sia-C7), 72.32 (Sia-C8), 73.72 (Sia-C6), 78.74 (C≡CH), 103.08 (Sia-C2), 122.60 (C≡CH), 122.94, 125.45, 128.64, 129.92, 153.88 (6C, C_6H_4), 172.83, 175.46 (2×CO).

4-{4-[3-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate-2-*O*-yl)-phenyl]-1H-(1,2,3) triazole-1-yl}-benzoic acid (**128**), (GG-2-001, *I-001*).

To a suspension of **124** (40.0 mg, 67.6 μmol) and 4-azidobenzoic acid **125** (11.0 mg, 67.6 μmol) in ^{t}BuOH (0.5 mL) and H_2O (0.5 mL) was added sodium ascorbate (6.7

μmol, 6.7 μL of a freshly prepared 1M solution in H_2O), followed by $CuSO_4 \cdot 5H_2O$ (0.170 mg, 0.676 μmol, in 5 μL of H_2O). The heterogeneous mixture was stirred vigorously at rt for 20 h, during which one additional portion of sodium ascorbate (6.7 μmol, 6.7 μL) and $CuSO_4 \cdot 5H_2O$ (0.170 mg, 0.676 μmol) were added according to TLC. Then diluted with DCM (10 mL), washed with H_2O (2×5 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (1% gradient of MeOH in DCM) to afford **128** (38.0 mg, 75%) as white foam.

$[\alpha]_D$ +13.9° (*c* = 0.73, CH_3OH); ^{1}H NMR (500 Hz, CD_3OD): δ 1.69 (s, 3H, CH_3-NHAc), 1.77, 1.83, 1.88, 1.93 (4s, 12H, 4×CH_3-OAc), 1.96 (m, 1H, Sia-H3a), 2.64 (dd, $J_{3a,3b}$ = 12.9, $J_{3b,4}$ = 4.7 Hz, 1H, Sia-H3b), 3.51 (s, 3H, OCH_3), 3.86 (t, $J_{4,5} = J_{5,6}$ = 10.5 Hz, 1H, Sia-H5), 3.97 (dd, $J_{8,9a}$ = 5.1, $J_{9a,9b}$ = 12.5 Hz, 1H, Sia-H9a), 4.14 (dd, $J_{8,9b}$ = 2.6, $J_{9a,9b}$ = 12.5 Hz, 1H, Sia-H9b), 4.28 (dd, $J_{5,6}$ = 10.8, $J_{6,7}$ = 1.2 Hz, 1H, Sia-H6), 5.19 (dd, $J_{6,7}$ = 1.4, $J_{7,8}$ = 8.8 Hz, 1H, Sia-H7), 5.24 (m,1H, Sia-H8), 6.94 (dd, *J* = 2.2, *J* = 8.2 Hz, 1H, Ar^1-H4), 7.19 (t, *J* = 8.0 Hz, 1H, Ar^1-H5), 7.42 (s, 1H, Ar^1-H2), 7.48 (d, *J* = 7.8 Hz, 1H, Ar^1-H6), 7.85, 8.04 (AA', BB' of AA'BB', *J* = 8.6 Hz, 2H, 4×Ar^3-CH), 8.81 (s, 1H, Ar^2-H5);

^{13}C NMR (125 MHz, CD_3OD): δ 20.65, 20.74, 20.80, 21.06 (4×CH_3-OAc), 22.70 (CH_3-NHAc), 39.32 (Sia-C3), 50.04 (Sia-C5), 53.53 (OCH_3), 63.17 (Sia-C9), 68.64 (Sia-C7), 70.14 (Sia-C4), 70.37 (Sia-C8), 74.14 (Sia-C6), 101.56 (Sia-C2), 118.99 (Ar^1-C2), 120.57 (Ar^2-C5), 120.94 (2C, Ar^3-C3, C5), 121.43 (Ar^1-C4), 122.63 (Ar^1-C6), 131.13 (Ar^1-C5), 132.47 (2C, Ar^3-C2, C6), 141.15, 149.12, 155.71 (5C, *ipso*-C), 169.29, 171.60, 171.85, 171.92, 172.49, 173.59 (7C, 7×CO);

Anal. Calcd for $C_{35}H_{38}N_4O_{15}$+2H_2O: C, 53.16; H, 5.35; N, 7.09. Found: C, 53.61; H, 5.20; N, 6.94.

Sodium 4-{4-[3-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate-2-*O*-yl)-phenyl]-1H-(1,2,3) triazole-1-yl}-benzoate (**120**), (GG-2-002, *II-002, 030*).

a) From **128**: to a solution of **128** (35.0 mg, 46.4 μmol) in MeOH (2.7 mL) was added freshly prepared 1M NaOMe/MeOH (0.3 mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified

by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **120** as a white solid (23.1 mg, 81%) after a final lyophilization from H_2O;

b) From **131**: to a suspension of **131** (8 mg, 19.0 μmol) and ethyl 4-azidobenzoyic acid (3.0 mg, 19.0 μmol) in ^{t}BuOH (0.2 mL) and H_2O (0.2 mL) was added sodium ascorbate (1.9 μmol, 1.9 μL of a freshly prepared 1M solution in H_2O), followed by $CuSO_4 \cdot 5H_2O$ (0.05 mg, 0.190 μmol, in 0.67 μL of H_2O). The heterogeneous mixture was stirred vigorously at rt for 16 h, and then concentrated by lyophilization. The resulting solid was purified by RP chromatography (5% gradient MeOH in H_2O) to afford **120** as a white solid (10.2 mg, 87%) after a final lyophilization from H_2O.

$[\alpha]_D$ +13.9° (*c* = 1.16, H_2O); ^{1}H NMR (500 Hz, D_2O): δ 1.96 (m, 1H, Sia-H3a), 2.04 (s, 3H, CH_3-NHAc), 2.92 (dd, $J_{3a,3b}$ = 12.5, $J_{3b,4}$ = 4.6 Hz, 1H, Sia-H3b), 3.60-3.64 (m, 2H, Sia-H7, Sia-H9a), 3.74-3.81 (m, 2H, Sia-H4, Sia-H9b), 3.89-3.97 (m, 3H, Sia-H5, Sia-H6, Sia-H8), 7.12-7.14, 7.37-7.40, 7.55-7.58, 7.59, 7.80-7.81, 8.00-8.02, 8.71 (m, 9H, 2×C_6H_4, C_2HN_3);

^{13}C NMR (125 MHz, D_2O): δ 23.38 (CH_3-NHAc), 41.25 (Sia-C3), 52.10 (Sia-C5), 62.88 (Sia-C9), 68.50, 68.55 (Sia-C4, Sia-C7), 72.26 (Sia-C8), 73.72 (Sia-C6), 103.08 (Sia-C2), 118.95, 120.70, 121.95, 121.98, 130.46, 130.48, 130.72, 138.27, 147.84, 154.63 (14C, 2×C_6H_4, C_2HN_3), 172.91, 175.40 (3C, 3×CO).

Ethyl {4-[3-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate-2-*O*-yl)-phenyl]-1H-(1,2,3) triazole-1-yl}-acetate (**129**), (GG-2-004, *II-004*).

To a suspension of **124** (20.0 mg, 33.8 μmol) and ethyl azidoacetate **126** (3.5 μL, 33.8 μmol) in ^{t}BuOH (0.15 mL) and H_2O (0.15 mL) was added sodium ascorbate (3.4 μmol, 3.4 μL of a freshly prepared 1M solution in H_2O), followed by $CuSO_4 \cdot 5H_2O$ (0.085 mg, 0.338 μmol, in 1.1 μL of H_2O). The heterogeneous mixture was stirred vigorously at rt for 20 h, during which three additional portions of sodium ascorbate (10.2 μmol, 10.2 μL) and $CuSO_4 \cdot 5H_2O$ (0.255 mg, 1.02 μmol) were added according to TLC. Then the mixture was diluted with DCM (10 mL), washed with H_2O (2×5 mL), and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (0.5% gradient of MeOH in DCM) to afford **129** (10.0 mg, 41%) as white foam.

$[\alpha]_D$ +6.3° (*c* = 0.92, $CHCl_3$); ^{1}H NMR (500 Hz, $CDCl_3$): δ 1.30 (X of ABX, *J*=7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.90 (s, 3H, CH_3-NHAc), 2.01, 2.03, 2.06, 2.11 (4s, 12H, 4×CH_3-OAc), 2.20 (t, $J_{3a,3b} = J_{3a,4} = 12.7$ Hz, 1H, Sia-H3a), 2.71 (dd, $J_{3a,3b} = 13.0$, $J_{3b,4} = 4.7$ Hz, 1H, Sia-H3b), 3.68 (s, 3H, OCH_3), 4.04 (q, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.3$ Hz, 1H, Sia-H5), 4.14 (dd, $J_{8,9a} = 5.8$, $J_{9a,9b} = 12.4$ Hz, 1H, Sia-H9a), 4.27 (A, B of ABX, *J* = 14.3, *J* = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 4.36-4.38 (m, 2H, Sia-H6, Sia-H9b), 5.10 (m, 1H, Sia-H4), 5.20, 5.24 (A, B of AB, *J* = 17.5 Hz, $C\underline{H}_2CO$), 5.32 (dd, $J_{6,7} = 1.8$, $J_{7,8} = 8.0$ Hz, 1H, Sia-H7), 5.35 (d, $J_{5,NH} = 10.0$ Hz, 1H, Sia-NH), 5.41 (ddd, $J_{7,8} = 8.2$, $J_{8,9a} = 5.8$, $J_{8,9b} = 2.9$ Hz, 1H, Sia-H8), 7.07 (m, 1H, Ar^1-H4), 7.33 (t, *J* = 8.0 Hz, 1H, Ar^1-H5), 7.50 (m, 1H, Ar^1-H2), 7.67 (d, *J* = 8.0 Hz, 1H, Ar^1-H6), 8.06 (s, 1H, Ar^2-H5);

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.05 ($CH_2\underline{C}H_3$), 20.65, 20.71, 20.83, 20.95 (4×CH_3-OAc), 23.18 (CH_3-NHAc), 37.67 (Sia-C3), 49.41 (Sia-C5), 50.86 ($\underline{C}H_2CO$), 53.01 (OCH_3), 62.21 (Sia-C9), 62.35 ($\underline{C}H_2CH_3$), 67.51 (Sia-C7), 68.75 (Sia-C4), 69.22 (Sia-C8), 73.25 (Sia-C6), 100.08 (Sia-C2), 118.33 (Ar^1-C2), 120.30 (Ar^1-C4), 121.62 (2C, Ar^1-C6, Ar^2-C5), 129.84 (Ar^1-C5), 131.64, 147.50, 153.74 (*ipso*-C), 166.31, 167.91, 170.08, 170.17, 170.22, 170.68, 170.91 (7×CO);

Anal. Calcd for $C_{32}H_{40}N_4O_{15}$: C, 53.33; H, 5.59; N, 7.77. Found: C, 53.54; H, 5.75; N, 7.45.

Sodium {4-[3-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate-2-*O*-yl)-phenyl]-1H-(1,2,3) triazole-1-yl}-acetate (**121**), (GG-GG-2-007, *II-007*).

To a solution of **129** (12.0 mg, 16.7 μmol) in MeOH (1.8 mL) was added freshly prepared 1M NaOMe/MeOH (0.2mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **121** as a white solid (9.00 mg, 98%) after a final lyophilization from H_2O.

$[\alpha]_D$ +13.8° (*c* = 0.75, H_2O); ^{1}H NMR (500 Hz, D_2O): δ 1.96 (m, 1H, Sia-H3a), 2.03 (s, 3H, CH_3-NHAc), 2.92 (m, 1H, Sia-H3b), 3.60-3.62 (m, 2H, Sia-H7, Sia-H9a), 3.75 (m, 1H, Sia-H4), 3.82 (m, 1H, Sia-H9b), 3.89-3.93 (m, 3H, Sia-H5, Sia-H6, Sia-H8),

5.08 (s, 2H, CH_2CO), 7.16 (d, J = 8.0 Hz, 1H, Ar^1-CH), 7.43 (t, J = 7.8 Hz, 1H, Ar^1-CH), 7.60-7.61 (m, 2H, Ar^1-H2, H6), 8.28 (s, 1H, Ar^2-H5);

^{13}C NMR (125 MHz, D_2O): δ 22.50 (CH_3-NHAc), 41.38 (Sia-C3), 52.22 (Sia-C5), 63.07 (Sia-C9), 68.61, 68.67 (Sia-C4, Sia-C7), 72.41 (Sia-C8), 73.87 (Sia-C6), 119.19, 121.88, 122.18, 130.62, 147.46, 150.08, 154.78 (8C, C_6H_4, C_2HN_3), 175.53 (3C, 3×CO).

1-Benzyl-{4-[3-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate-2-*O*-yl)]-phenyl}-1H-[1,2,3] triazole (130), (GG-2-005, *II-005*).

To a suspension of **124** (34.0 mg, 57.5 μmol) and benzyl azide **127** (7.2 μL, 57.5 μmol) in ^{t}BuOH (0.2 mL) and H_2O (0.2 mL) was added sodium ascorbate (5.7 μmol, 5.7 μL of a freshly prepared 1M solution in H_2O), followed by $CuSO_4 \cdot 5H_2O$ (0.140 mg, 0.57 μmol, in 1.9 μL of H_2O). The heterogeneous mixture was stirred vigorously at rt for 20 h, during which nine additional portions of sodium ascorbate (51.3 μmol, 51.3 μL) and $CuSO_4 \cdot 5H_2O$ (1.26 mg, 5.13 μmol) were added according to TLC. Then the mixture was diluted with DCM (10 mL), washed with H_2O (2×5 mL) and dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography (0.5% gradient of MeOH in DCM) to afford **130** (28.4 mg, 68%) as white foam.

$[\alpha]_D$ +6.8° (c = 0.95, $CHCl_3$); ^{1}H NMR (500 Hz, $CDCl_3$): δ 1.90 (s, 3H, CH_3-NHAc), 2.01, 2.02, 2.03, 2.11 (4s, 12H, 4×CH_3-OAc), 2.19 (m, 1H, Sia-H3a), 2.70 (dd, $J_{3a,3b}$ = 13.0, $J_{3b,4}$ = 4.7 Hz, 1H, Sia-H3b), 3.67 (s, 3H, OCH_3), 4.05 (q, $J_{4,5} = J_{5,6} = J_{5,NH}$ = 10.3 Hz, 1H, Sia-H5), 4.13 (dd, $J_{8,9a}$ = 5.6, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9a), 4.33 (dd, $J_{8,9b}$ = 2.8, $J_{9a,9b}$ = 12.4 Hz, 1H, Sia-H9b), 4.36 (dd, $J_{5,6}$ = 10.8, $J_{6,7}$ = 1.8 Hz, 1H, Sia-H6), 4.98 (ddd, 1H, $J_{3a,4}$ = 12.0, $J_{3b,4}$ = 4.7, $J_{4,5}$ = 10.5 Hz, 1H, Sia-H4), 5.33 (dd, $J_{6,7}$ = 1.8, $J_{7,8}$ = 7.9 Hz, 1H, Sia-H7), 5.34-5.41 (m, 2H, Sia-H8, Sia-NH), 5.56, 5.60 (A, B of AB, J = 14.9 Hz, 2H, Ph$\underline{C}H_2$), 7.05-7.07, 7.30-7.38, 7.39, 7.45-7.61 (m, 9H, C_6H_4, C_6H_5), 7.83 (s, 1H, C_2HN_3);

^{13}C NMR (125 MHz, $CDCl_3$): δ 20.60. 20.71, 20.82, 20.94 (4×CH_3-OAc), 23.17 (CH_3-NHAc), 37.70 (Sia-C3), 49.38 (Sia-C5), 52.98 (OCH_3), 54.16 (Ph$\underline{C}H_2$), 62.09 (Sia-C9), 67.43 (Sia-C7), 68.77 (Sia-C4), 69.25 (Sia-C8), 73.25 (Sia-C6), 100.08 (Sia-C2), 118.08, 120.02, 120.09, 121.56, 128.09, 128.73, 129.08, 129.80, 131.84,

134.74, 147.51, 153.81 (14C, C_6H_4, C_2HN_3, C_6H_5), 167.89, 170.05, 170.22, 170.63, 170.90 (6C, 6×CO);

Anal. Calcd for $C_{35}H_{40}N_4O_{13}$+1/2H_2O: C, 57.29; H, 5.63; N, 7.64. Found: C, 57.20; H, 5.60; N, 7.22.

1-Benzyl-{4-[3-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate-2-*O*-yl)]-phenyl}-1H-[1,2,3] triazole (**122**), (GG-2-009, *II-009,028*).

a) From **130**: to a solution of **130** (25.6 mg, 35.3 μmol) in MeOH (2.25 mL) was added freshly prepared 1M NaOMe/MeOH (0.25mL). The mixture was stirred at rt for 5 h under argon, then H_2O (0.5 mL) was added and the mixture was stirred at rt for 16 h. The solution was neutralized by 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), Dowex ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **122** as a white solid (17.3 mg, 87%) after a final lyophilization from H_2O,

b) From **131**: to a suspension of **131** (10 mg, 23.2 μmol) and benzyl azide (2.9 μL, 23.2 μmol) in ^{t}BuOH (0.2 mL) and H_2O (0.2 mL) was added sodium ascorbate (2.3 μmol, 2.3 μL of a freshly prepared 1M solution in H_2O), followed by $CuSO_4 \cdot 5H_2O$ (0.06 mg, 0.232 μmol, in 0.8 μL of H_2O). The heterogeneous mixture was stirred vigorously at rt for 16 h, and then concentrated by lyophilization. The resulting solid was purified by RP chromatography (5% gradient MeOH in H_2O) to afford **122** as a white solid (11.7 mg, 89%) after a final lyophilization from H_2O.

$[\alpha]_D$ +15.6° (*c* = 0.58, H_2O); ^{1}H NMR (500 Hz, D_2O): δ 1.95 (t, $J_{3a,3b} = J_{3a,4} = 12.2$ Hz, 1H, Sia-H3a), 2.03 (s, 3H, CH_3-NHAc), 2.91 (dd, $J_{3a,3b} = 12.5$, $J_{3a,4} = 4.7$ Hz, 1H, Sia-H3b), 3.53 (dd, $J_{8,9a} = 5.8$, $J_{9a,9b} = 12.0$ Hz, 1H, Sia-H9a), 3.61 (dd, $J_{6,7} = 1.6$, $J_{7,8} = 9.2$ Hz, 1H, Sia-H7), 3.67 (dd, $J_{8,9b} = 2.4$, $J_{9a,9b} = 12.0$ Hz, 1H, Sia-H9b), 3.75 (ddd, $J_{3a,4} = 11.9$, $J_{3b,4} = 4.7$, $J_{4,5} = 9.5$ Hz, 1H, Sia-H4), 3.82 (ddd, $J_{7,8} = 8.7$, $J_{8,9a} = 5.8$, $J_{8,9b} = 2.4$ Hz, 1H, Sia-H8), 3.87 (dd, $J_{5,6} = 10.4$, $J_{6,7} = 1.7$ Hz, 1H, Sia-H6), 3.91 (q, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.4$ Hz, 1H, Sia-H5), 5.56 (s, 2H, PhC$\underline{H}_2$), 7.11-7.13, 7.32-7.42, 7.48-7.51 (m, 9H, C_6H_4, C_6H_5), 8.21 (s, 1H, Ar^2-H5);

^{13}C NMR (125 MHz, D_2O): δ 22.18, (CH_3-NHAc), 41.03 (Sia-C3), 51.91 (Sia-C5), 54.10 (Ph$\underline{C}H_2$), 62.57 (Sia-C9), 68.24, 68.29 (Sia-C4, Sia-C7), 72.01 (Sia-C8), 73.53 (Sia-C6), 102.86 (Sia-C2), 118.91, 121.70, 121.73, 122.45, 128.16, 128.89, 129.27,

130.27, 130.59, 134.96, 147.33, 154.50 (14C, C_6H_4, C_6H_5, C_2HN_3), 172.68, 175.24 (2×CO).

Ethyl {4-[3-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosynate-2-*O*-yl)-phenyl]-1H-[1,2,3] triazole-1-yl}-acetate (**132**), (GG-2-029, *II-029*).

To a suspension of **131** (10 mg, 23.2 μmol) and ethyl azidoacetate **126** (2.4 μL, 23.2 μmol) in ^{t}BuOH (0.2 mL) and H_2O (0.2 mL) was added sodium ascorbate (2.3 μmol, 2.3 μL of a freshly prepared 1M solution in H_2O), followed by $CuSO_4 \cdot 5H_2O$ (0.06 mg, 0.232 μmol, in 0.8 μL of H_2O). The heterogeneous mixture was stirred vigorously at rt for 16 h, and then concentrated by lyophilization. The resulting solid was purified by RP chromatography (5% gradient MeOH in H_2O) to afford **132** as a white solid (12.0 mg, 93%) after a final lyophilization from H_2O.

$[\alpha]_D$ +18.4° (*c* = 0.68, H_2O); ^{1}H NMR (500 Hz, D_2O): δ 1.28 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 1.97 (t, $J_{3a,3b} = J_{3a,4} = 12.2$ Hz, 1H, Sia-H3a), 2.04 (s, 3H, CH_3-NHAc), 2.92 (dd, $J_{3a,3b} = 12.5$, $J_{3b,4} = 4.6$ Hz, 1H, Sia-H3b), 3.60-3.64 (m, 2H, Sia-H7, Sia-H9a), 3.76 (m, 1H, Sia-H4), 3.79 (dd, $J_{8,9b} = 2.1$, $J_{9a,9b} = 12.0$ Hz, 1H, Sia-H9b), 3.88-3.95 (m, 3H, Sia-H5, Sia-H6, Sia-H8), 4.27, 4.30 (A, B of ABX, J = 7.1 Hz, $C\underline{H}_2CH_3$), 5.40 (s, 2H, NCH_2), 7.16-7.18, 7.42-7.45, 7.59-7.61, 8.34 (m, 5H, C_6H_4, C_2HN_3);

^{13}C NMR (125 MHz, D_2O): δ 13.56 ($CH_2\underline{C}H_3$), 22.39 (CH_3-NHAc), 41.31 (Sia-C3), 52.50 (N-CH_2), 52.12 (Sia-C5), 63.01 (Sia-C9), 63.74 ($\underline{C}H_2CH_3$), 68.48, 68.56 (Sia-C4, Sia-C7), 72.37 (Sia-C8), 73.78 (Sia-C6), 103.12 (Sia-C2), 119.03, 122.01, 124.00, 130.56, 130.72, 147.56, 154.70 (8C, C_6H_4, C_2HN_3), 169.10 (3C, 3×CO).

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Publications:

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