Exploring The Carbohydrate-Binding Sites of Myelin-associated Glycoprotein (MAG) and Its Ligands by a Integrated Dynamic Approach

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Dekan

To,

My Lovely FAMILY

-

TAI, BABA and PUPPY

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Declaration

I declare, that I wrote this thesis "Exploring The Carbohydrate-Binding Sites of Myelin-associated Glycoprotein (MAG) and Its Ligands By A Dynamic Integrated Approach" 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.

Sachin Vilasrao Shelke,

Basel, June 2006

Abbreviations

BSA	bovine serum albumin
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
PNS	peripheral nervous system
DRG	dorsal root ganglion
FDP	fluorescein diphosphate
GPI	glycosylphosphatidylinositol
IC50	concentration required for 50% inhibition
LRR	leucine rich repeat
MAG	Myelin-associated glycoprotein
MAG-/-	MAG-deficient
MAG+/+	MAG-wild type
OMgp	Oligodendrocyte-myelin glycoprotein
NgR	Nogo receptor
R118A	mutation of Arg118 to Ala
R118D	mutation of Arg118 to Asp
RG	retinal ganglion
siglec	sialic acid binding immunoglobulin-like lectin

sLeX	sialyl lewisX
SMP	Schwann cell myelin protein
Sn	sialoadhesion
rIP	relative inhibitory potency
Ac	acetyl
Bn	benzyl
Bz	benzoyl
CSA	camphor sulfonic acid
DCE	dichloroethane
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	N, N'-dimethylformamide
DMTST	dimethyl(methylthio)sulfonium triflate
ESI-MS	electrospray ionization mass spectrometry
Fuc	fucose
Gal	galactose
GalNAc	N-acetyl galactosamine
Glc	glucose
i-PrOH	2-propanol
KD	dissociation constant
kDa	kiloDalton
KDN	5-deaminated neuraminic acid
Ki	inhibitory constant
logD7.4	n-octanol/water partition coefficient at pH 7.4

Neu5Ac	*N*-acetylneuraminic acid
NeuGc	*N*-glycolyl neuraminic acid
NgR	Nogo receptor
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
OSE	2-(trimethylsilyl)ethyl
PE	petrolether
PND	post-natal day
PNS	peripheral nervous system
py	pyridine
RP	reversed phase
SAR	structure-activity relationship
Sia	sialic acid
STD	saturation transfer difference
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid, triflic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
TsOH	*p*-toluenesulfonic acid
PPh$_3$	Triphenylphosphine
SA	Sialic acid
NI	5-Nitro Indole
TEMPO	2,2,6,6-tetramethyl-1-piperidine-1-oxyl
NaN$_3$	Sodium azide

CONTENTS

<u>INTRODUCTION</u>

Hieroglyphs of the ancient Egyptians provide the earliest known reference to the fact that, unlike the peripheral nervous system (PNS) the adult mammalian central nervous system (CNS) does not spontaneously regenerate after injury. The Edwin Smith surgical papyrus, which dates back to 1550 BC, states that "If you examine a man with a neck injury … and find he is without sensation in both arms and both legs, and unable to move them, and he is incontinent of urine … it is due to the breaking of the spinal cord caused by dislocation of a cervical vertebra. This is a condition which cannot be treated".

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]

What happens when there is a spinal cord injury (SCI)? There are two types of SCI, (a) *Tetraplegics*: Suffer impairment or loss in the arms as well as in the trunk, legs and pelvic organs and (b) *Paraplegics*: Have impairment or loss if motor and/or sensory function in the trunk, legs and pelvic organs, but have retained some or all of this fuction in the region of arms.

The known therapie is to either immobilizing the spine, a surgery and a steroid

treatment or rehabilitation. However to date there is no cure for SCI.

1. Inhibition of axonal regeneration in adult mammalian CNS:

There are two main obstacles to regeneration after injury: inhibitors within the myelin sheets and formation of glial scars (**figure 1**).[6] The scars are formed by reactive astrocytes, which change their morphology to act as a physical barrier to axonal outgrowth and also upregulate several extracellular-matrix-associated inhibitors after injury.[7] However, since the glial scar formation 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.[8] 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.[9] The myelin sheath is interrupted at regular intervals by the nodes of Ranvier that are relatively small, unmyelinated regions of the axon. 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.[10] 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*.[11-15] 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]
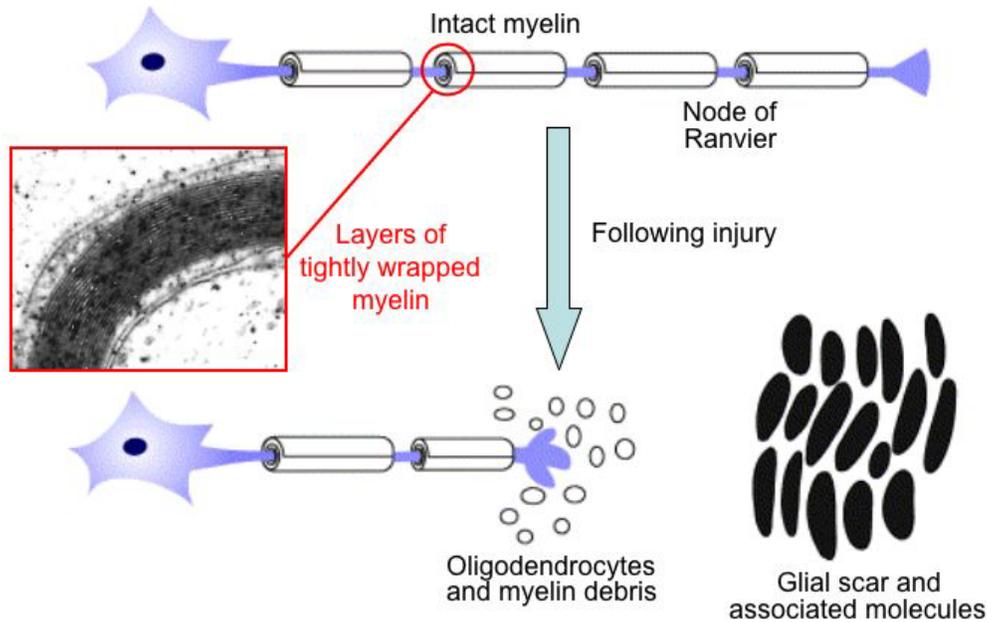
Figure 1:

Why does the 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. However 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] In contrast, 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.

1.1. Inhibitors of regeneration in myelin:

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.

1.1.1. Nogo

Schwab and his colleagues in the late 1980s gave a molecular insight into the mechanism of inhibition.[20,21] The identification of IN-1 monoclonal antibody, which was raised against an inhibitory fraction of myelin A was the first indication that specific molecules in myelin were involved in neurite outgrowth. It allowed the axons to grow on myelin as shown both *in vitro*[21] and *in vivo*.[1] After a decade later, an antigen for the IN-1 antibody was cloned independently by three groups, which was named Nogo by Schwab for its inhibitory action on axonal growth (**figure 2**).[22-24] Nogo belongs to the reticulon family, and is expressed as the distinct isoforms A, B, and C in CNS, but not in PNS.[23] Among all three isoforms, Nogo-A is the only one that is expressed in oligodendrocytes, and therefore has been studied extensively. 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 intracellular surface, and amino-Nogo on the extracellular 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] Recombinant Nogo-A is recognized by the monoclonal antibody IN-1, and it inhibits neurite outgrowth from dorsal root ganglion (DRG) and spreading of 3T3 fibroblasts in an IN-1 sensitive manner,[22] which showed that Nogo-A is a potent inhibitor of neurite outgrowth.
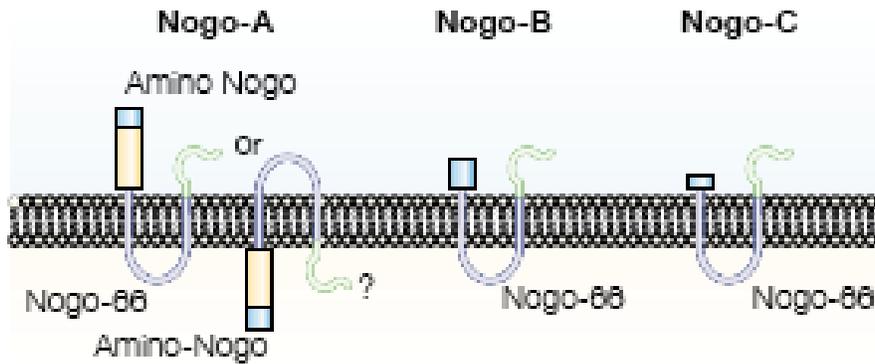
Figure 2: Nogo-A, B, and C.

1.1.2. Myelin-associated glycoprotein

Myelin-associated glycoprotein (MAG, **figure 3**), ˜10 kDa glycoprotein which was described in 1973,[25] was the first protein of myelin to be characterized as an inhibitor of axonal outgrowth.[26,27] It is a member of the siglec (*s*ialic acid binding *i*mmuno*g*lobulin-like *lec*tin) family[28] and contains five Ig-like domains in its extracellular sequence. The first Ig-domain adopts an unusual conformation by folding over the second Ig-domain. It has 8 to 9 potential *N*-linked glycosylation sites and consists of typically 30% carbohydrate by weight.[29] It exists in two alternatively spliced isoforms, a large (L) and a small (S) form that differ only in their cytoplasmic sequences.[30-32] In the CNS, MAG is located in the periaxonal myelin membrane, while in the PNS, it is found in the outermost membrane of the myelin sheath.[33,34]

McKerracher *et al.*[26] first observed MAGs inhibitory character of axonal regeneration by employing octyl glucoside to extract myelin from the CNS. After a chromatographic purification using a diethylaminoethyl (DEAE) anion exchange column, MAG was found to be a major component of the multiple neurite growth inhibitory proteins present in myelin of the CNS.
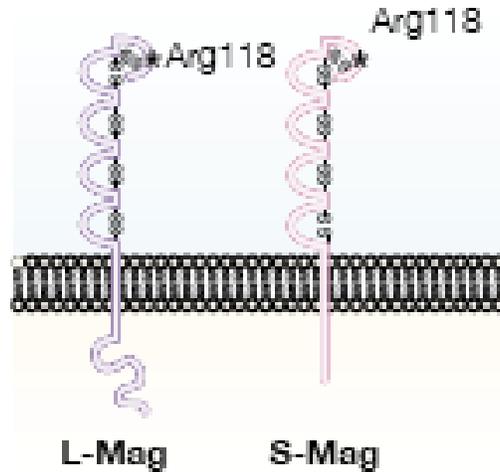
Figure 3: L-MAG and S-MAG.

Interestingly, neurons early in their cell cycle are not inhibited by MAG, in fact their growth is promoted in most cases.[27,35,36] Therefore, there is evidence that points to MAG is a bifunctional molecule, since all neurons studied to date switch their response to MAG from promotion to inhibition, depending on age and type. 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. A number of neurons respond to MAG by switching from promotion to inhibition during development.[27,37] For retinal ganglion (RG) neurons and spinal neurons, the switch occurs by birth, while for dorsal root ganglion (DRG) neurons, the transition takes place sharply at post-natal day 3 (PND 3).

To further elucidate the inhibitory role of MAG, MAG-/- mice were created by deletion of the MAG gene by homologus recombination.[38,39] Surprisingly, tests both *in vitro* and *in vivo* showed no significant difference of axonal regeneration between MAG-/- and MAG+/+ mice.[40] 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. In addition, it is possible that the effect of inhibitory molecules on regeneration is not additive; therefore the presence of any one inhibitor may be sufficient to prevent most regenerations.

As MAG is a sialic acid-binding protein, it specifically binds to sialo-glycoproteins and sialo-glycolipids (gangliosides). The Arg118 from the first Ig-domain of MAG was recently identified to be crucial for its sialic acid binding capability. When Arg118 of MAG is mutated, its sialic acid-binding capability is lost and as well as its ability to inhibit neurite outgrowth.[41] Surprisingly, when a 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.

1.1.3. Oligodendrocyte-myelin glycoprotein

The existence of oligodendrocyte-myelin glycoprotein (OMgp, **figure 4**) was also known long before it was shown to be an inhibitor of axonal regeneration.[42] 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.[43] 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.[44] OMgp contains a leucine rich repeat (LRR) domain, followed by a *C*-terminal domain with serine/threonine repeats. *In vitro* Omgp induces growth cone collapse and potentially inhibits neurite outgrowth, but it's function *in vivo* has not yet been reported.[43]
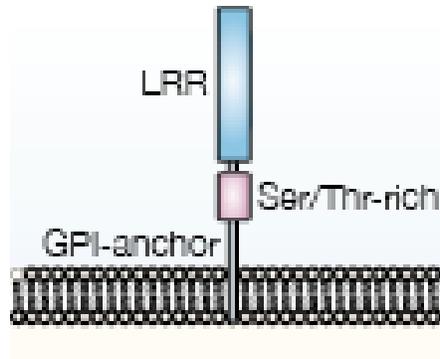
Figure 4: Oligodendrocyte-myelin glycoprotein (OMgp).

1.2. Receptors for Myelin-associated inhibitors of axonal growth

After the identification of these inhibitors, the next key step was to understand how axons respond to inhibitory influences. In particular, the receptors that transduce their inhibitory signals across the membrane to the neuron should be identified. In 2001, Strittmatter *et al.* cloned a binding partner of Nogo-66 which was termed as Nogo receptor (NgR).[45] 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.[45]

One of the most surprising and interesting findings was the discovery that NgR can also bind and mediate the inhibitory activity of MAG and OMgp.[43,44,46] 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] These observations suggest that this peptide could interfere with the ability of MAG and OMgp to bind to NgR.[7]

As NgR is a GPI-linked protein it has no transmembrane or cytoplasmic domain. Consequently, even though NgR is essential for Nogo-66, MAG and OMgp to exert their inhibitory effects, it cannot tansduce the signal across the membrane

and depends on the support of a partner. Even before the discovery of NgR as a common receptor for all three inhibitors, the p75 neutrotropin receptor (p75^{NTR}) was reported as the tranducer for MAG.[48] The low-affinity p75^{NTR} was identified as a co-receptor of NgR.[49,50] 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.[51,52] 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 over expressed in primary neurons, attenuates the same set of inhibitory activities, suggesting that p75^{NTR} is a transducer of inhibitory signals into the cytosol of responding neurons.[49]
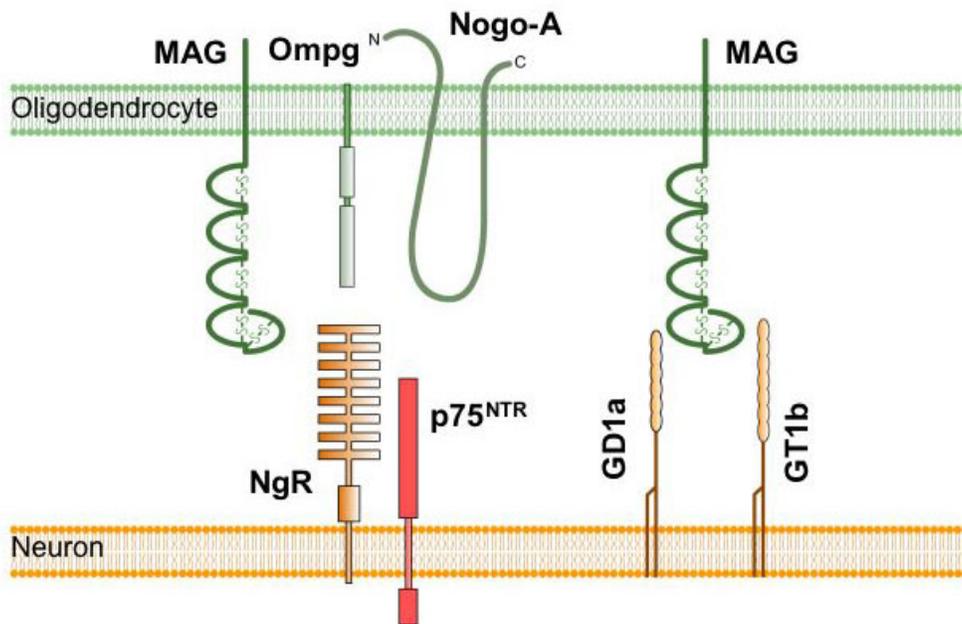
Figure 5: Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS.

After studying all the molecular aspects of the inhibitory effects of myelin, MAG, Nogo and OMgp were identified as the three main myelin-associated inhibitors which interact with the same receptor complex NgR-p75^{NTR} to transduce the inhibitory signal across the membrane (**figure 5**). The transduced signal initiates a signaling cascade leading to the inhibition of axonal outgrowth after injury.[53] However, the postulated model implies that there is functional redundancy

between these inhibitors. This idea of redundancy could 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,[54] and none at all in another.[24] 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 axon regrowth.[26,55,56] 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.[57]

2. Gangliosides as functional ligands for MAG

It has long been assumed that MAG mediates neurite outgrowth inhibition by the interaction with gangliosides (**figure 5**).[53] However, the role of gangliosides was questioned after NgR was identified as the receptor of MAG. One opinion is that gangliosides can only potentiate and augment MAG-mediated inhibition of neurite outgrowth by facilitating the clustering of signaling molecules.[6] In contrast many lines of evidence supports the hypothesis that the nerve cell surface gangliosides are specific functional ligands responsible for MAG-mediated neurite outgrowth inhibition.[10,57,58]

Gangliosides, sialic acid-bearing glycosphingolipids, are the major glycans of nerve cells and the major sialic acid-containing glycoconjugates 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 previously mentioned, MAG belongs to the siglecs, a structurally and functionally related family of cell surface receptors that bind to sialic acid containing glycoproteins and glycolipids.[28] 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 alone or the V-set domain and 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$\rightarrow$3)Galβ(1$\rightarrow$3)Gal*N*Ac terminus as implicated by initial specificity

study.[57] As already reported 30 years ago, gangliosides carry 75-80% of the sialic acids content in the brain.[59] Representative examples are the major brain gangliosides GD1a and GT1b (**Figure 6**). In addition, these gangliosides embedded in the neuronal cell surface and also along the axons and 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 therefore may mediate MAGs physiological functions.
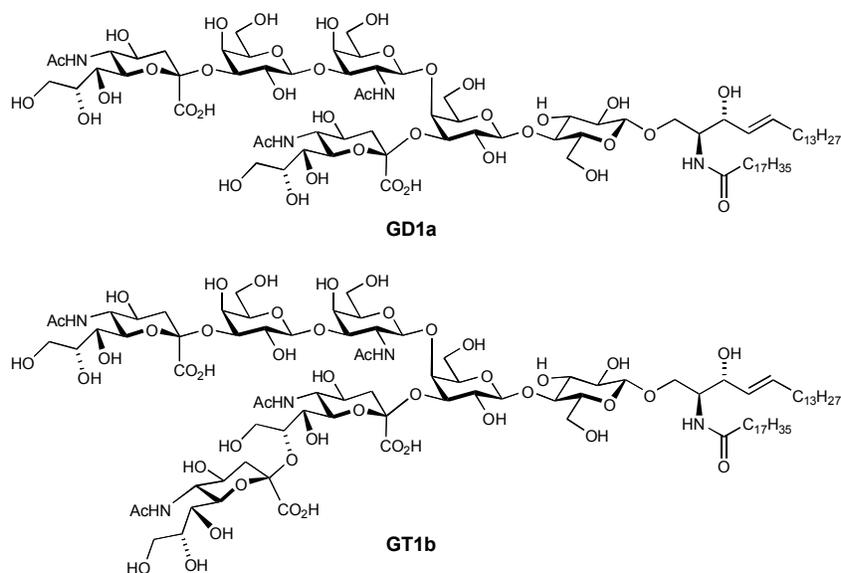
Figure 6: major brain gangliosides GD1a, GT1b.

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.[58] In short gangliosides containing the Neu5Acα(2→3) Galβ(1→3)GalNAc terminus, are not only 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],[48] implicating that, besides MAG-NgR-P75[NTR], gangliosides and P75[NTR] may form a second type of receptor complex with MAG to transmit the inhibitory signals into neurons.

2.1. Structural specificities of gangliosides for MAG binding

Mammalian ganglioside-binding proteins have distinct structural specificities for their carbohydrate targets.[57] To date, a number of structure-function studies of MAG-mediated cell adhesion to gangliosides and related glycosphingolipids have been performed to elucidate structural specificities of MAG-recognized carbohydrate (**Table 2**). [57,71,73]

Several points are note worthy upon consideration of the data in **Table 2**. First, it was observed that MAG only binds to gangliosides containing a terminal α-2,3-linked sialic acid (compare GM1 to GD1a, GD1b to GT1b, *entries 2 to 7 and 8 to 11*), but the adhesion was abrogated when it is capped by a α-2,8-linked sialic acid (compare GT1β to GQ1β, GT1b to GQ1b, *entries 10 to 12 and 11 to 13*).[57]

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)-GalNAc-β(1-4)-Gal-β(1-4)-Glc-β-Cer | Neu5Ac-α(2-3) **GM1**	n.d.[b]	57,73

3	Neu5Ac-α(2-6) 丨 Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **GM1α**	n.d.	[57,73]
4	**Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **GM1b**	80	[57,73]
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) 丨 **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **GD1α**	19	[57,73]
7	**Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer 丨 Neu5Ac-α(2-3)　**GD1a**	50	[57,73]
8	Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer 丨 Neu5Ac-α(2-8)-Neu5Ac-α(2-3)　**GD1b**	n.d.	[71]
9	Neu5Ac-α(2-6) 丨 **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer 丨 Neu5Ac-α(2-3)　**GT1aα**	17	[57,73]
10	Neu5Ac-α(2-8)-Neu5Ac-α(2-6) 丨 **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer **GT1β**	50	[57]
11	**Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer 丨 Neu5Ac-α(2-8)-Neu5Ac-α(2-3)　**GT1b**	50	[57,73]
12	Neu5Ac-α(2-8)-Neu5Ac-α(2-6) 丨 Neu5Ac-α(2-3)-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer 丨 Neu5Ac-α(2-8)　**GQ1β**	n.d.	[57]
13	Neu5Ac-α(2-3)-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer 丨　　　　　　　　　丨 Neu5Ac-α(2-8)　Neu5Ac-α(2-8)-Neu5Ac-α(2-3)　**GQ1b**	n.d.	[57]
14	Neu5Ac-α(2-6) 丨 **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer 丨 Neu5Ac-α(2-8)-Neu5Ac-α(2-3)　**GQ1bα**	6.0	[57,73]
15	**Neu5Ac-α(2-3)**-Gal-β(1-3)-**Glc*N*Ac**-β(1-4)-Gal-β(1-4)-Glc-β-Cer	240	[57,73]
16	**Neu5Ac-α(2-3)**-Gal-β(1-**6**)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer	87	[57,73]
17	**HO₃S-(6)** 丨 **Neu5Ac-α(2-3)**-Gal-β(1-3)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer	22	[57,73]
18	**HO₃S-(6)** 丨 **Neu5Ac-α(2-3)**-Gal-β(1-**4**)-Gal*N*Ac-β(1-4)-Gal-β(1-4)-Glc-β-Cer 丨 **HO₃S-(3)**	1.5	[57,73]

[a] low but statistically significant adhesion over background.

[b] no detectable adhesion at >100 pmol/well.

Secondly, it is apparent from Table 2 that all but one member of an unique quantitatively minor family of gangliosides termed "Chol-1" gangliosides, GQ1bα (*entry 14*), displays enhanced potency for MAG-mediated adhesion except GM1α (entry 3).[73] Chol-1 gangliosides are related to the major brain gangliosides, but have an additional α2,6-linked sialic acid on the Gal*N*Ac moiety of the gangliotetraose core, making them part of the "α-series" ganglioside family.[74-76] 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).[74-76] 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 detectable affinity).[57,73]

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*).[57,73] Furthermore, the different affinity between GD1α and GD1a suggests that as an additional contributor, α-2,6-linked sialic acid is more significant than α-2,3-linked internal sialic acid, although both of them can enhance MAG-mediated adhesion (*entries 6 and 7*).[57,73]

Finally, 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.[57] Surprisingly, altering the Gal-Gal*N*Ac linkage from β-1,3 to β-1,6 (*entry 16*), which might be expected to have a large conformational effect, did not influence MAG binding by comparing with GM1b (entry 4).[57] This is consistent with a study by Kelm *et al.*[77] 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. These data indicate 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.

In summary, the α-2,3-linked sialic acid on the terminal Gal is the primary determinant for MAG binding, while the additional α-2,6-linked sialic acid on Gal*N*Ac can measurably increase the binding affinity. Compared with the significant impacts of these two sialic acids, the disaccharide core is not comparably specific, which implies that it may be not greatly involved in the protein-binding site. According to the observations from the above SAR 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 major pharmacophore for MAG binding (**figure 7, in box**).
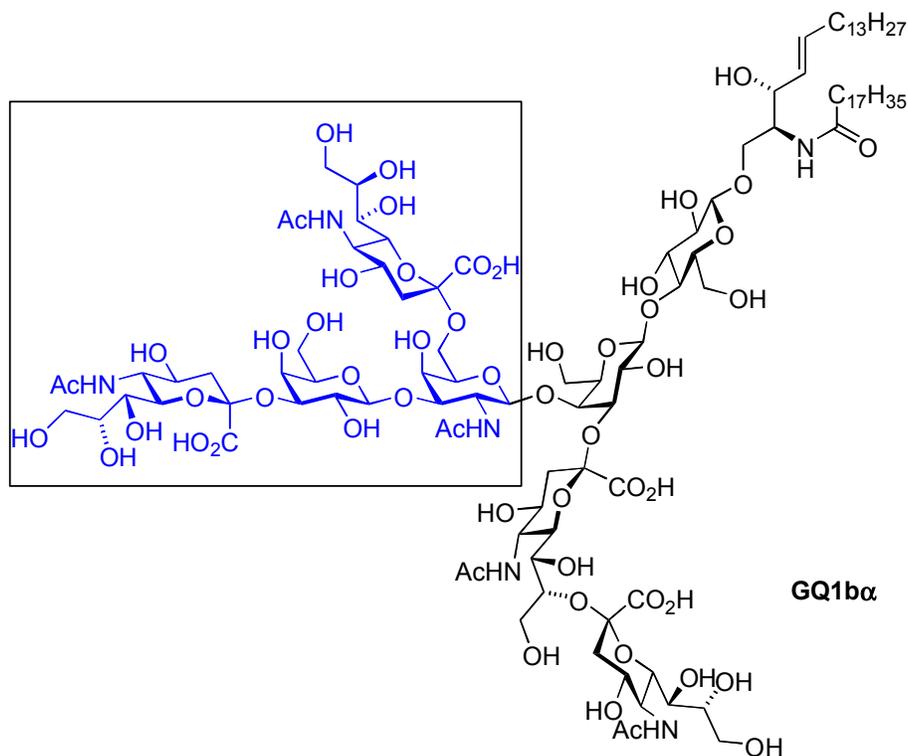
Figure 7: Ganglioside GQ1bα with tetrasaccharide "Neu5Acα(2→3)Galβ(1→3)[Neu5Acα(2→6)] Gal*N*Ac" in box.

2.2. Functional groups of sialic acid interacting with MAG

As previously discussed, the sialic acids of gangliosides are essential for MAG-binding. Sialic acids occur on cell surfaces at exposed positions, mostly as terminal components with different linkages to the neutral core 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.[78,79].

Sialic acids are the most complex monosaccharides in vertebrate glycoconjugates. They constitute a family of structures based on *N*-acetylneuraminic acid, the most abundant sialic acid and precusor to diverse structures bearing modifications on the glycerol side chain, and/or hydroxyl at C-4. To further investigate the contributions of substructural features of sialic acid for MAG-binding affinity, chemically synthesized and modified gangliosides were employed for an extensive structure-function study.

Schnaar *et al.*[57,58,71,73,80] studied the 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.*[77,81] employed a "hapten inhibition assay" to observe 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.

Since GD1a significantly supports adhesion of MAG, it was used as a basis for testing sialic acid modifications. Modifications of the carboxylic acid by esterification, amidation, or reduction, abolishe the MAG-binding, strongly demonstrating that the anionic charge is crucial for protein binding.[71] Additionally,

this carboxylic acid needs to be in axial position, since only the α-anomer, the natural conformation in sialylated glycans supports MAG-binding.[81] This orientation 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. The structure shows the axial carboxylate of the terminal sialic acid forming a salt bridge with Arg97.[82] It should be noted that this Arg is highly conserved in the first Ig-domain of other members of the siglec family (Arg97 in sialoadhesin corresponds to Arg118 in MAG).[41]

The glycerol side chain of sialic acid was shown by Schnaar to be crucial for binding with a modified GD1a, whereas the binding affinity to MAG was abolished with truncated glycerol chain, 7/8-aldehydes, 7/8-alcohols, as well as deoxy and/or methoxy derivatives of the 7, 8 or 9-position of Sia(I) of GM3.[71,80] These results suggest that the intact glycerol chain is needed for binding and that eliminating or derivatizing any of its hydroxyls significantly diminishes binding. The role of the 9-OH attracts interest based on the X-ray of sialoadhesin co-crystallized with sialyllactose, which implicates that a hydrogen bridge with a backbone carbonyl of the protein might exist.[82]

From Kelm's study of a series of methyl sialosides (**Table 3**),[81] additional structural information was obtained demonstrating the requirement of 9-OH for binding (**Figure 8**). Replacement of 9-OH with hydrogen (*entry 3*) or halogens (*entries 4 to 6*) reduced affinity, while a $9\text{-}NH_2$-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*), either due to the weaker hydrogen bonds formed by –SH with the protein, or due to the steric constraints by the large sulfur atom. More recently, a systematic study based on $C(9)\text{-}NH_2$ was carried out by differently substituting the amine.[83]

●	Neu5AcαMe (1)
■	9-deoxy-Neu5AcαMe (3)
▲	9-Iodo-Neu5AcαMe (6)
○	9-NH$_2$-Neu5AcαMe (8)
□	9-Chloro-Neu5AcαMe (4)
△	9-Thio-Neu5AcαMe (7)
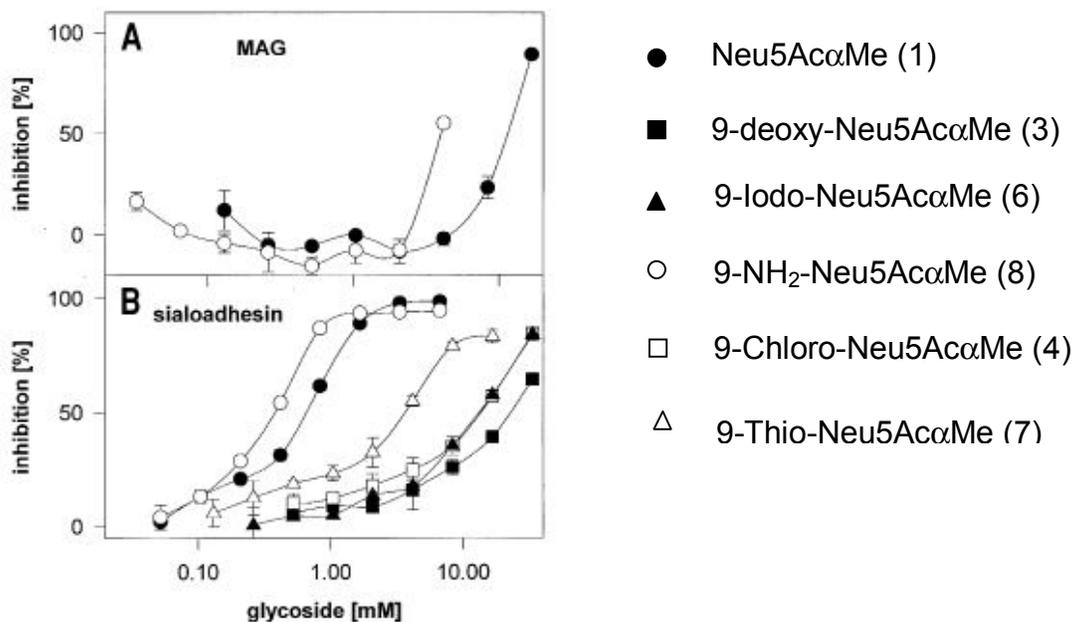
Figure 8: The influence of substituents at C-9 of sialic acid.

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.[84]

Based on the X-ray structure of sialoadhesin co-crystallized with sialyllactose, which showed that a Trp residue in the binding site interacts specifically with the methyl group of the *N*-acetyl moiety, Kelm *et al.*[81] synthesized derivatives to investigate this position in greater detail (**Figure 9**).

This analysis confirmed the crucial role of *N*-acetyl group for recognition (**Table 3**). Halogenated acetyl groups showed a significant enhancement of binding to MAG. In Kelm's study, the acetyl residue was replaced with propionyl (*entry 12*), aminoacetyl (*entry 13*), thioacetyl (*entry 14*) or halogenated acetyl groups (*entries 15-17*). Introducing a chlorine atom (*entry 16*) or a trifluoromethyl group (*entry 17*) enhanced the affinity for MAG by 7- and 4-fold, respectively. It should

be noted that *N*-fluoroacetyl sialic acid derivative (*entry 15*) binds nearly 17-fold better than the reference.

● Neu5**Ac**αMe (1)

■ Neu5**thio**αMe (14)

○ Neu5**prop**αMe (12)

□ Neu5**NH₂**αMe (13)
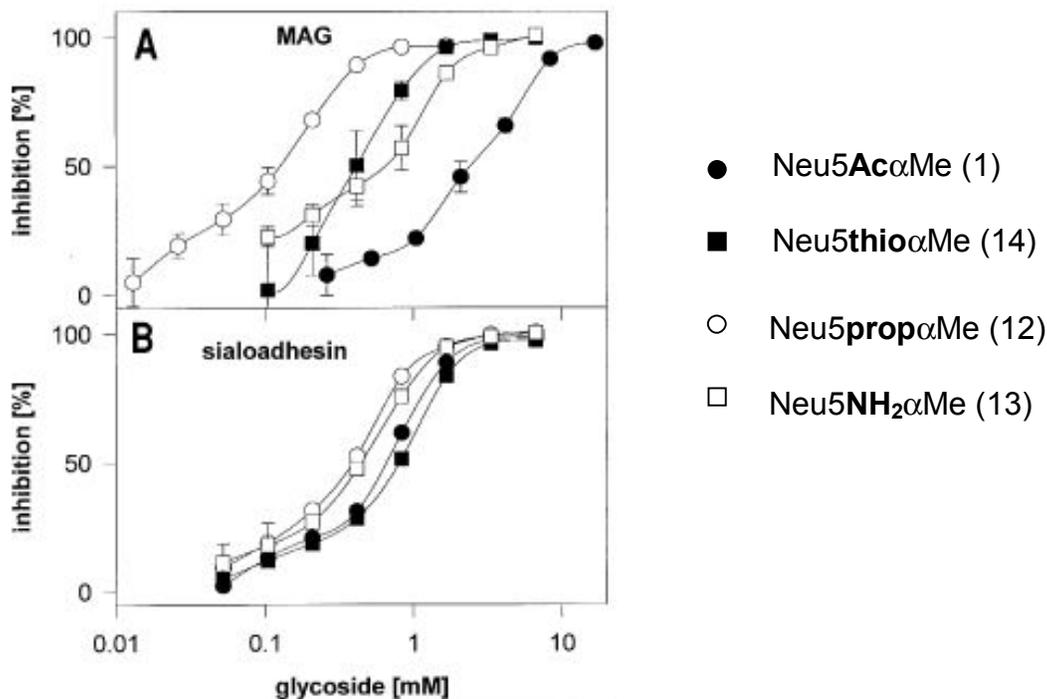
Figure 9: The influence of substituents at C-5 of sialic acid.

The enhanced affinity can be rationalized by additional halogen mediated contacts with the protein or by electronic effects on the amide. This would result in a weaker hydrogen bond acceptor quality of the carbonyl oxygen and a significantly increased hydrogen bond donor quality of the amino group. [81]

Table 3: Various substituted methyl sialosides.

Entry	Compound	rIP	[Ref.]
1	Neu5Ac-α-Me	1.00	81
2	Neu5Ac-α-Bn	9.80	81
3	9-deoxy-Neu5Ac-α-Me	n.a.	81
4	9-Cl-Neu5Ac-α-Me	n.a.	81
5	9-Br-Neu5Ac-α-Me	n.a.	81

6	9-I-Neu5Ac-α-Me	n.a.	[81]
7	9-thio-Neu5Ac-α-Me	n.a.	[81]
8	9-NH$_2$-Neu5Ac-α-Me	2.98	[81]
9	9-benzoyl-NH-Neu5Ac-α-Me	704	[83]
10	9-bipheyl-4-carbonyl-NH-Neu5Ac-α-Me	218	[83]
11	9-naphthyl-2-carbonyl-NH-Neu5Ac-α-Me	236	[83]
12	Neu5Propyl-α-Me	1.56	[81]
13	Neu5aminoAc-α-Me	n.a.	[81]
14	Neu5ThioAc-α-Me	3.85	[81]
15	Neu5FAc-α-Me	16.94	[81]
16	Neu5ClAc-α-Me	7.00	[81]
17	Neu5F$_3$Ac-α-Me	4.04	[81]

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

Based on the above SAR studies it was concluded that a hydroxy group at C-9 and the *N*-acetyl residue at C-5 are essential structural features of for the binding of Sia by members of the siglec family (**Figure 10**).
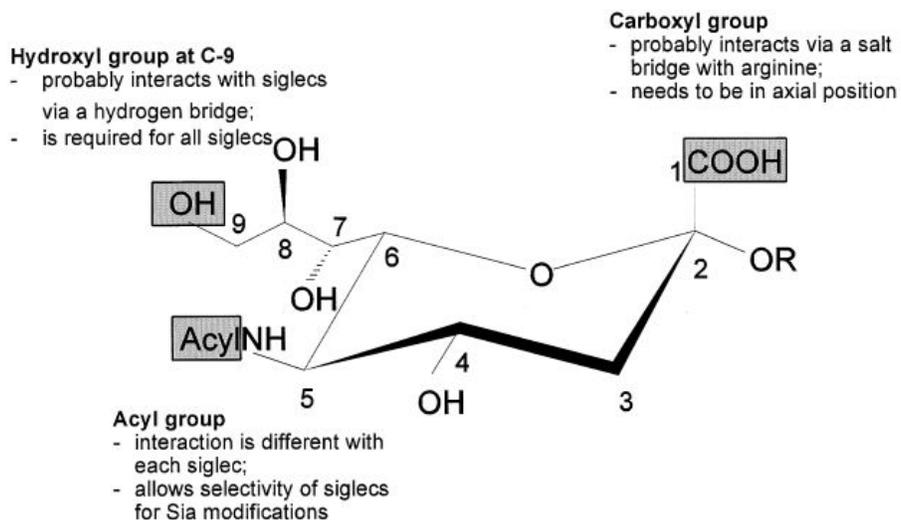
Figure 10: Functional groups of sialic acid interacting with siglecs

3. Carbohydrates Based Drug Discovery

Traditionally, carbohydrates were considered to be solely of use for energy storage or as skeletal components. However this hypothesis was challenged in 1963 when a protein was isolated from *Canavalia ensiformis* with a demonstrated ability to bind to carbohydrates on erythrocytes. In the mean time carbohydrates became a major focus of biological research. As ligands for 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.[85] As a result, the development of carbohydrate-based therapeutics[86] appears to have great potential for the treatment of a myriad of human diseases.

Carbohydrates have long been avoided by chemists and biologists because of their structural complexity, which makes them difficult to analyze and extremely difficult to synthesize. Now, recent advances in the synthetic tools offered by carbohydrate chemistry and in the understanding of their biological role, have put sugar-based drugs on medicine's menu, where they are garnering impressive early reviews. Carbohydrate compounds are currently in clinical trials aimed at targeting numerous therapeutic areas including inflammation, tissue rejection, hepatitis and cancer.

3.1. Carbohydrate-based therapeutics

The carbohydrate binding proteins have been termed as "lectins" and are found in varying densities on all cell-surface membranes.[87-89] The lectins interact specifically with oligosaccharides and glycoconjugates on surrounding cells via H-bonding, metal coordination, Van der Waal's forces and hydrophobic interactions.[90,91] It is believed that favourable interactions between the hydroxyl groups of carbohydrates and the amino groups of the proteins aid molecular

recognition processes. These interactions are relatively weak, but their cumulative effects are so numerous that specific interactions occur.

Diseases where carbohydrate-based drugs are making an impact include cancer, diabetes, AIDS; influenza, bacterial infections and rheumatoid arthritis.[92] The carbohydrate-based therapeutics can be rationalized in following terms;

3.1.1. Inhibition of carbohydrate-lectin interactions

Many lectins on the surface of bacteria show specific binding to carbohydrates expressed on human cells and such interactions form an essential part of the infection pathway. Moreover, microbial enzymes can modify carbohydrate chains on host cells, resulting in an increased surface density of lectin ligands, which can enhance a bacterium's virulence. For example, *Pseudomonas aeruginosa* (a respiratory virus) produces the enzyme neuraminidase in the lungs of cystic fibrosis patients, which cleaves sialic acid from a glycolipid,[93] unmasking a carbohydrate receptor for the pathogen.

Soluble forms of human cell surface oligosaccharide components are being investigated and developed for rational anti-infective drug design. Such compounds are excellent drug candidates as they are small (approximately 1 kDa) and non-immunogenic. Anti-infective agents that are used clinically, or are undergoing clinical trials, include kanamycin (**Figure 11**), are of great interest when resistance to other antibiotics is observed. An analogue of kanamycin, dibekacin, has anti-tuberculosis properties as well as a broad spectrum of antimicrobial activity. Arbekacin, an aminoglycoside antibiotic that is currently on the market, has antibacterial activity against both Gram-positive and Gram-negative bacteria and is stable in the presence of aminoglycoside-inactivating enzymes produced by methicillin resistant *Staphylococcus aureus* (MRSA).
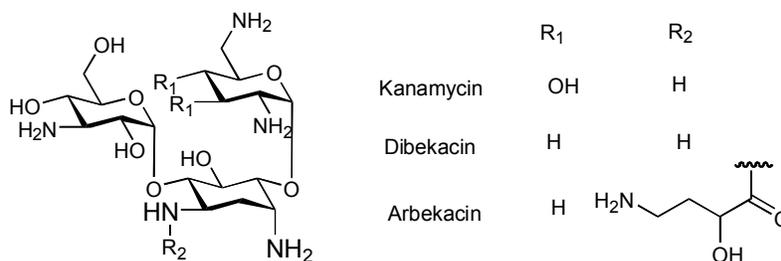
Figure 11: Structure of kanamycin, dibekacin and arbekacin.

Although lectin–carbohydrate interactions are essential for an efficient immune system, a more pernicious role is in the progression and metastasis of cancer cells.[94,95] In clinical trials aimed at inhibiting metastasis have been reported that the administration of sialyl Lewis[x] mimetics that occupy the selectin binding sites offer a great therapeutic potential since they block the selectin–carbohydrate binding interactions (**Figure 12**).
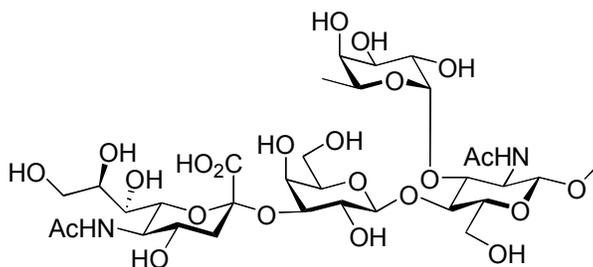
Figure 12: Structure of Sialyl Lewis[x].

3.1.2. Inhibition of enzymes that synthesize disease-associated carbohydrates

carbohydrates research has received particular attention for their potential to inhibit tumour growth and metastasis. The assembly of carbohydrates within biological systems occurs in the golgi apparatus and involves a number of glycosidase (carbohydrate trimming enzymes)- and glycosyl transferase (carbohydrate transfer enzymes)-mediated steps. For example, the biosynthesis of the cancer-associated sialyl Lewis[x] tetrasaccharide is accomplished by three glycosyl transferase enzymes. It has been demonstrated that carbohydrate analogues, that mimic their shape and electronic properties in transition state,

are capable of inhibiting specific enzymes involved in carbohydrate biosynthesis, offering the potential to disrupt the synthesis of disease-associated carbohydrates. As a result, interactions between the carbohydrates and the lectins would be disrupted and the disease will be unable to progress in the normal manner.

Some examples of inhibitors of carbohydrate biosynthesis that show promise as therapeutic agents are shown in **Figure 13**. Examples of particular interest are highlighted in **Table 4**.

Nojirimycin

Castanospermine

(-)-Swainosine

(+)-1-Deoxynojirimycin

Isofagomine

Salacinol

Deoxymannonojirimycin

Mannonojirimycin
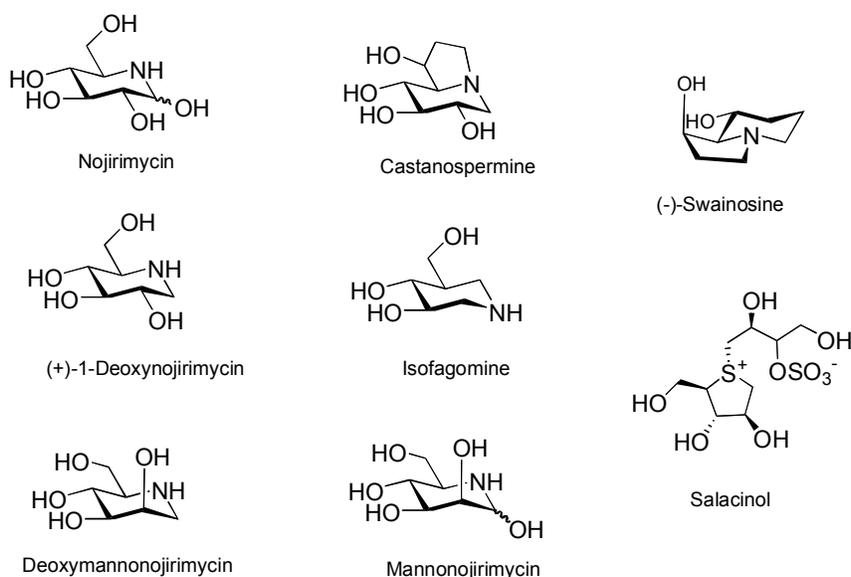
Figure 13: Some naturally occurring glycosidase (carbohydrate trimming enzyme) inhibitors.

Table 4: Some aza-carbohydrate drugs under clinical investigation.

Drug (Code)	Target	Company
Swainsonine (GD0039)	Renal, colorectal, breast cancer	Glycodesign
Vevesca 15 (OGT 918) (Zavesca)	Fabry's, Gaucher's disease, HIV	Oxford Glycoscience
Celgosivir (MDL 28574, DRG-0202, BuCast)	HIV/AIDS	Hoechst Marion Roussel

Swainsonine, is currently in phase II clinical trials for cancer therapy. It is

administered orally and has been shown to reduce solid tumours and haematological malignancies.[96,97] Zavesca (Vevesca) (**Figure 14**) is an orally active imino sugar that is a potent inhibitor of glucosylceramide glucosyltransferase, the enzyme responsible for converting ceramide to glucosylceramide during glycosphingolipid (GSL) biosynthesis.

Celgosivir (6-*O*-Butanoylcastanospermine)   Zavesca (*N*-Butyl-Deoxynojirimycin)
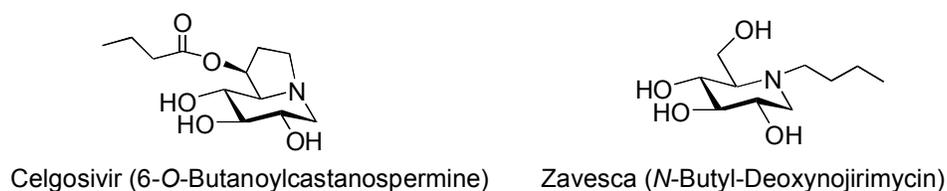
Figure 14: Structure of some aza-sugar drugs.

Diabetics can also benefit from administration of glycosidase inhibitors and a number of carbohydrate-derived therapies have been developed (**Figure 15, Table 5**). For example, acarbose is an α-glucosidase inhibitor blocking the digestion of carbohydrates in the small intestine.[98]

Miglitol

Acarbose
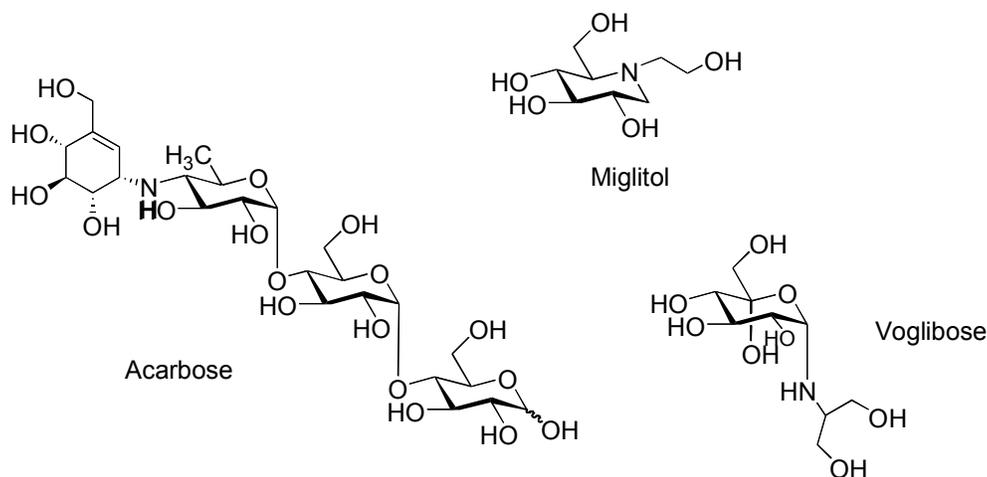
Voglibose

Figure 15: Structure of some current anti-diabetic drugs.

Table 5: Some anti-diabetic drugs.

Drug (Code)	Target	Company
Acarbose (Glucobay,Precose, Prandase)	Diabetes (Type I and II)	Bayer AG
Voglibose (AO-128, Basen, Glustat)	Diabetes (Type II)	Takeda/Abbott
Miglitol (BAY M 1099, Glyset)	Diabetes	Bayer

Carbohydrate analogues have also been developed as potential treatments for influenza (**Figure 16**). As mentioned previously, the influenza virus possesses surface proteins that complex with specific membrane-bound oligosaccharides on human cells. Neuraminidase protrudes from the surface of the influenza virus The glycomimetic drugs bind to the neuraminidase, which stops the virus from exiting the host cell, reducing the amount of virus that is released to infect other cells.

N-Acetyl neuraminic acid
Sialic acid

Zanamivir (Relenza)

Peramivir

Oseltamivir (Tamiflu)
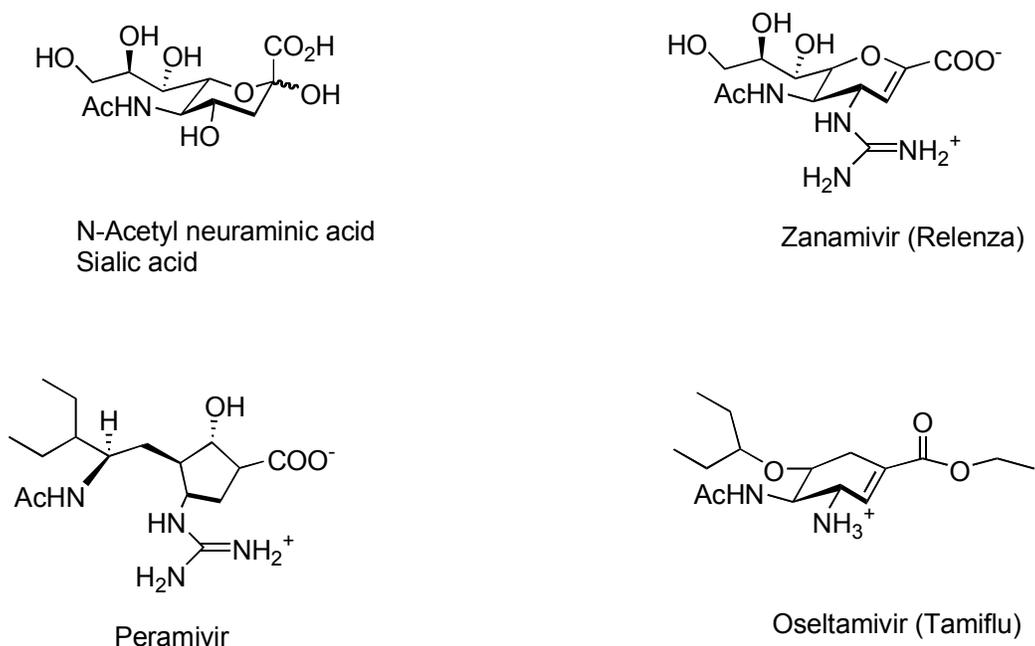
Figure 16: Structure of some anti-influenza drugs.

Zanamivir is a transistion state mimic that selectively inhibits influenza A and B virus neuraminidases, which are essential for the release of the virus from infected host cells.[99] A recent review describes the development of new anti-influenza drugs.[100]

3.1.3. Carbohydrate-based anti-thrombotic agents

Heparin was the first polysaccharide-based drug to find widespread use in man since 1937 for the treatment of thrombosis. It is isolated from mammalian tissues as a complex mixture of glycosaminoglycans (GAG) and demonstrates powerful anticoagulant activity. It enhances the ability of antithrombin to inactivate thrombin and factor Xa, which are enzymes that promote coagulation. A number of carbohydrate-based drugs have been developed via chemical[101] and

enzymatic[102] methods as alternative anti-thrombotic agents. For example, **Table 6** portrays low-molecular-weight heparin-based drugs that have been approved for the treatment of thrombosis.

Table 6: Examples of low-molecular-weight heparin-based drugs currently on the market

Drug	Target	Company
Dalteparin Sodium (Fragmin)	Thrombosis, anticoagulant	Pfizer
Nadroparin calcium (Fraxiparin)	Thrombosis, anticoagulant	Sanofi-Synthelabo
Enoxaparin sodium (Clexane)	Thrombosis, anticoagulant	Aventis
Ardeparin (Normiflo)	Thrombosis, anticoagulant	Wyeth
Danaparoid (Organan)	Thrombosis, anticoagulant	Organon
Fondaparinux (Arixtra)	Thrombosis, anticoagulant	Organon/Sanofi-Synthelabo

3.2. Drawbacks of carbohydrates as drugs:

In spite of the structural diversity and the broad range of biological functions, there have been only a relatively small efforts in developing new mimetics or drugs. There are series of obstacles that make carbohydrates a problematic class of lead structures for drug development, specifically their complicated synthetic availability, poor bioavailability, and low binding affinity for target proteins.[103-105]

3.2.1. Synthetic difficulties

Due to the structural complexity resulting from various regio- and stereospecific linkages of oligosaccharides, carbohydrates are rarely chosen as lead structures. 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, the synthesis of specific glycosidic linkages is much more difficult, as carbohydrates are densely functionalized with hydroxyl groups of similar reactivity. Cubersome protection and deprotection steps leads to synthese with a large number of synthetic steps. Despite recent

advances in solid phase synthesis,[106-108] efficient regio- and stereoselective synthese of oligosaccharides remains a considerable challenge.

3.2.2. Low bioavailability

Many natural saccharides are rapidly degraded *in vivo*. Most glycosides are hydrolyzed either under the acidic conditions in the stomach or by glycosidases in the small intestine.[109] This is the reason that oligosaccharides are generally not orally available. In addition, various glycosidases are present in body fluids, which limits a carbohydrate-based drugs plasma half-life to just a few minutes, depending on its structure.[105] Finally, the hydrophilic character of carbohydrate leads to their fast renal excretion.

3.2.3. Low binding affinity

Carbohydrate-protein interactions show typically low affinities, where the dissociate constant is most often in the mM to μM range ($K_D \approx 10^{-3}$ to 10^{-6} M). By contrast the general requirements for pharmaceutical applications is much lower ($K_D \approx 10^{-9}$ M).[110] Despite their low-affinity, the binding processes that carbohydrates display are highly specific. 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 are rather small and have been extensively debated.[110-112] The lack of hydrophobic groups in carbohydrate moieties, which are often dominant in high-affinity receptor-ligand interactions is also responsible for their low affinity.

3.3. Carbohydrate mimics

To overcome these drawbacks, various strategies and approaches were applied. Research on multivalent carbohydrate ligands that increase the affinity through multiple simultaneous binding events has been reported.[113-116] In addition

numerous studies have been devoted to developing and optimizing carbohydrate mimics.[103,117] There are two main strategies for the design of carbohydrate mimics.

- The substitution of the *O*-glycosidic bond with non-natural linkages or replacement of ring oxygen by N, S or C.[118]
- The replacement of the entire glycosidic scaffold by a small, non-carbohydrate scaffold bearing the required functional groups in the same spatial orientation as the parent sugar structure.[119]

3.3.1. O-glycosidic bond substituted mimics

For mimics such as *C*-glycosides, *S*-glycosides or carba-sugars, substitution would ideally increase the resistance to endogenous enzymatic hydrolysis and chemical degradation, while conserving the global geometry of the native oligosaccharide.[118]

3.3.2. Mimics based on non-carbohydrate scaffolds

These type of mimics are generally based on different core structures, while maintaining the functions of the carbohydrate lead structure by retaining 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.[103]

An example of the strategy described above is demonstrated with **CGP69669**, a mimic of the tetrasaccharide sLe^x, a natural ligand for E-selectin (**Figure 17**). **CGP69669** was designed based on the information that anionic charge is the only pharmacophore of the terminal sialic acid and the Glc*N*Ac behaves merely as a spacer to hold Gal and Fuc in the appropriate spatial orientation. For this mimic, 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 a *in vitro* biological assay.[120]

sLe^x; IC_{50} = 1mM

CGP 69669; IC_{50} = 0.08mM
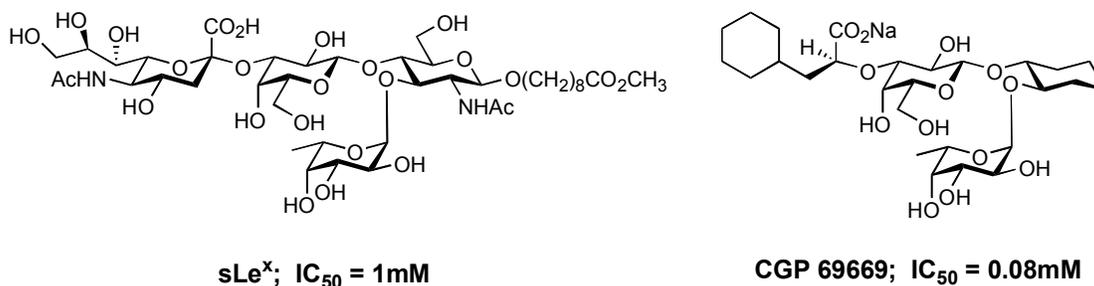
Figure 17: sLe^X and its mimic **CGP69669.**

As mentioned earlier, another famous example is the development of anti-influenza agents (**Figure 18**).[121] Zanamivir was designed to mimic the transition state of sialic acid (Neu5Ac2en) in the hydrolysis reaction by neuraminidase. The carboxylic acid, glycerol side chain and *N*-acetyl functions that are essential for the binding were retained. In addition the basic guanidinyl group was attached on 4-position, offering the formation of an optimal salt bridge with Glu 119 and Glu 227 identified by X-ray crystal analysis.[121] Notably, K_i of Zanamivir is 4,000 times better than the parent compound Neu5Ac2en.[122] However, due to its poor bioavailability of only 2% ($logD_{7.4} \approx$ -6), Zanamivir cannot be applied orally and was developed as a nasal spray. 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. This mimic is now on the market as an orally administered drug to block influenza virus infection.[123-125]

Neu5Ac2en K_i Å1μM

Zanamivir K_i Å1nM

Oseltamivir K_i Å1nM
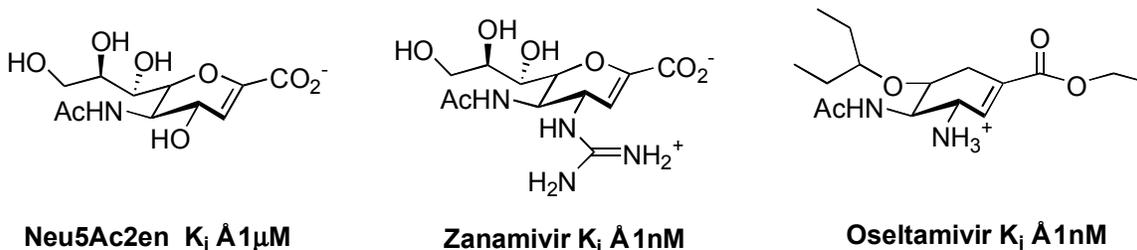
Figure 18: Neu5Ac2en, Zanamivir and Oseltamivir at physiological pH**.**

4. 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 of MAG (Fc-MAG$_{d1-3}$ or Fc-MAG$_{d1-5}$) was produced in COS cell and purified.[77,81] The fluorescent hapten inhibition assay (*figure* 19) employed in this project is a successor of the previous one which was based on radio-labeled MAG and erythrocytes as target cells.[77,81]
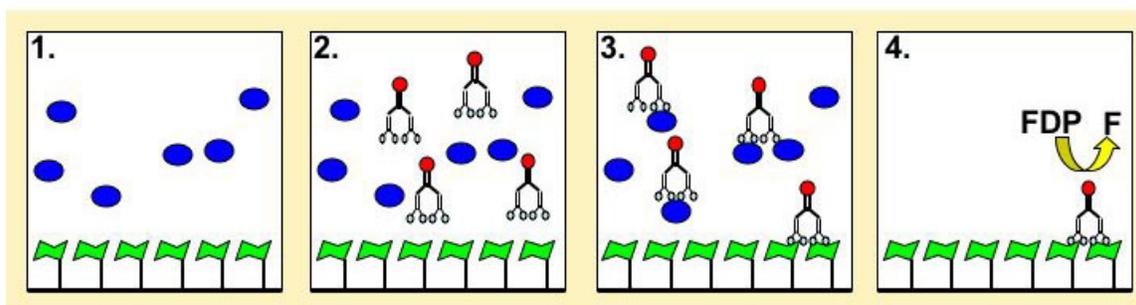
Figure 19: Fluorescent hapten inhibition assay. **1**. The inhibitor is added to the sialic acid coated microtitre plates; **2**. Fc-MAG complexed with polyclonal *goat anti human* IgG(Fc) coupled with alkaline phosphatase is added; **3**. After incubation, unbound complexes are removed; **4**. By treating with fluorescein diphosphate (FDP), the resulting fluorescence intensity that is proportional to the amount of bound complexes can be determined.

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$_{50}$ may vary depending on the condition from assay to assay, relative inhibitory potencies (rIPs) are calculated by dividing the IC$_{50}$ of the reference compound by the test compound.[77,81] It is notable that rIP values are consistent, thus allowed a reliable comparison among different assays as long as the same reference is used.

$$\text{rIP} = \frac{\text{IC}_{50} \text{ (reference compound)}}{\text{IC}_{50} \text{ (analyte)}}$$

RESULTS AND DISCUSSION

5. Sialic acid derivatives modified at 2 and 9-position

Kelm *et* al. reported that Neu5AcαBn has a ten-fold higher affinity for MAG than the corresponding methyl glycoside.[81] Substituting the 9-hydroxy group of sialic acid by an amino group enhances binding, while substitution by halogens decreases the binding drastically, suggesting the necessity of a hydrogen donor at the 9-position. It was also shown that, introducing an amide group at 9-position of sialic acid increases the binding to MAG dramatically.[126,83] Based on these results, several benzyl sialosides (**Scheme 1**) were synthesized with a wide range of amide residues at the 9-position.

Starting from neuraminic acid **1**, sialyl donor **3** was synthesized according to a reported procedure.[127] The reaction of compound **3** with benzyl alcohol in the presence of NIS/TfOH yielded the isomeric mixture of benzyl sialosides **4α** (41 %) and **4β** (18 %).[128] Subsequent saponification of **4α** and **4β** gave the benzyl sialosides **5α** and **5β**. *O*-Deacetylation of **4α** (→**6**) followed by selective *p*-tosylation at 9-position afforded compound **7**.[129] The azido group was introduced using sodium azide and crown ether 18-C-6.[130] Subsequent saponification of azide **8** afforded the sodium salt **10**. Acetylation of **8** using acetic anhydride and pyridine gave compound **9**. Amidation with a wide range of aromatic and aliphatic acyl chlorides under modified Staudinger conditions[131] afforded the 9-amido-substituted sialosides **11a-g** in 54%-70% yeild. Final saponification of yielded the target substances **12a-g** for the bioassay studies.
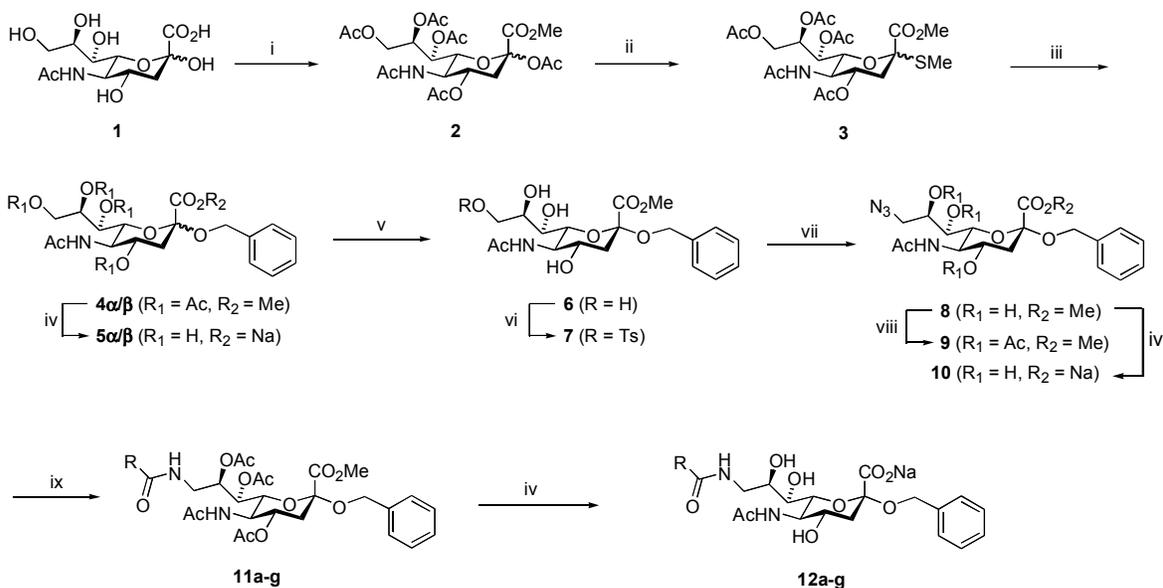
Scheme 1: i) a. MeOH, Amberlyst 15, b. Ac$_2$O, DMAP, pyr, 81%; ii) TMSSMe, TMSOTf, MS 3Å, DCE 82%; iii) PhCH$_2$OH, NIS/TfOH MeCN, 41% α, 18 % β; iv) a. 10% NaOH, MeOH, b. Dowex 50X8 (Na$^+$), 70-95%; v) NaOMe, MeOH, 2 h, 82%; vi) *p*-TsCl, pyr, 56%; vii) NaN$_3$, DMF, 18-C-6, 75%; viii) Ac$_2$O, DMAP, pyr, 73%; ix) RCOCl, PPh$_3$, DCM, 54-70%.

The inhibitory potencies of the benzyl sialosides **5α**, **10** and **12a-g** (**Table 7**) towards MAG were measured using a fluorescent hapten inhibition assay.[77,81] (see p45)

The inhibition assay indicated that the introduction of amide group at 9-position strongly increased the affinity compared to that of the 9-hydroxy sialoside **5α**. Another interesting observation was that, the aromatic moieties (compounds **12a-c**) were found to be significantly more active than the aliphatic moieties (compounds **12e-g**). Compound **12a** showed the most promising affinity for MAG with a rIP of 690. The insertion of sterically more demanding biphenyls (**12b** and **12c**) or a hydrophilic orotinoyl residue (**12d**) led to a decrease in the affinity. The observation that 9-azido compound **10** turned out to be inactive may be due to the lack of a hydrogen donor at 9-position.
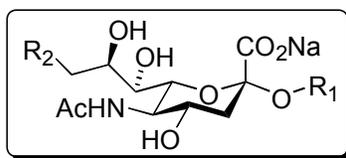
Table 7: Relative inhibitory potencies (rIPs) of sialosides with respect to trisaccharide **A**

Entry	Compound	R_1	R_2	rIP
1	**A**	Gal-β(1-3)-GalNAc-OSE	OH	1
2	**5α**	OBn	OH	0.6
4	**10**	OBn	N$_3$	0.05
5	**12a**	OBn		690
6	**12b**	OBn		165
7	**12c**	OBn		68
8	**12d**	OBn		6
9	**12e**	OBn		10
10	**12f**	OBn		12
11	**12g**	OBn		9

The relative inhibitory potencies (rIPs) were calculated by dividing the IC$_{50}$ of the reference compound by the IC$_{50}$ of the compound of interest. The higher the rIP, stronger the binding of compound. Trisaccharide **A** was choosen as a reference compound due to its easy availability via chemo-enzymatic synthesis.[132]

After optimizing the substituent at the 9-position of sialic acid and achieving a higher affinity, we decided to explore the influence of a benzamido substituent on the activity of the reference compound **A.** Towards this end trisaccharide **B** was synthesized and tested against the reference compound **A**.

In addition, benzyl ether functionality at C-9 position (**C**) was introduced to evaluate the necessity of an amide group for the higher activity. This group is more flexible than the amide group while the phenyl is retained for possible hydrophobic interactions.

Using the fluorescent hapten inhibition assay, compounds **B** and **C** were tested together with compound **A** as a reference. The resulting rIPs are listed in **Table 8**, which also includes compounds **12a** and **5α** for comparison. According to the rIP values, trisaccharide **B** with the benzamido substituent at the 9-position of the sialic acid residue showed a 203-fold enhanced affinity over the reference compound **A**. However, **B** was approximately 3 fold weaker than its monosaccharide analogue **12a** with a benzyl group as aglycon instead of the disaccharide core. This finding was surprising since previous studies demonstrated the existence of additional contacts of the subterminal saccharides with the protein.[81] It is possible that, this dramatical improvement in affinity of **12a** might be caused by "induced fit" of the protein on binding. This line of reasoning may also explain the low binding affinity of compound **B**, in which the disaccharide core is hindered from binding by the change of the protein conformation. It was also seen that monosaccharide **C** had only 3.7 times higher affinity than **5α**. This demonstrates that the effect of the substitution by benzyl ether is not comparable to the dramatic enhancement by a benzoylamide replacement of 9-hydroxy seen with **12a**. It is likely that the hydrogen bond interaction is crucial for 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.

Table 8: Relative inhibitory potencies (rIPs) of mimics with C-9 modified sialic acid:

Entry	Compound	Structure	rIP	rIC$_{50}$ (μM)
1	**12a**		690	0.001
2	**5α**		0.62	1.61
3	**C^x**		2.3	0.43
4	**B^x**		203	0.004
5	**A^y**		1	1
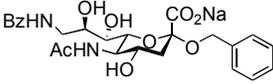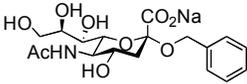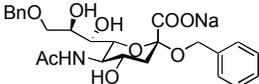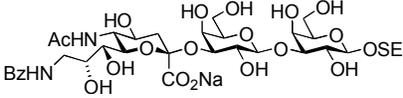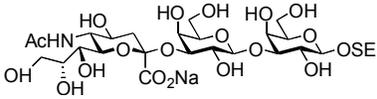
(**x**) Synthesized by Dr. Ganpan Gao at the Institute of Molecular Pharmacy, University of Basel

(**y**) Synthesized by Dr. Oliver Schwardt at the Institute of Molecular Pharmacy, University of Basel

6. 2-*O*-Benzyl Sialic Acid derivatives modified at C-9 position

6.1. Topliss operational scheme:

The rIP's indicated that the aromatic substitution at the 9-position was more beneficial for increasing the affinity for MAG compared to aliphatic substitution (**Table 7**). Using the Topliss Operational Scheme (TOS) a systematic investigation of the influence of aromatic substitution on the inhibitory activity of sialic acid analogues was therefore initiated.[133] The TOS is a non-mathematical application of the Hansch analysis, designed to guide the synthesis towards the

most active analogue of a lead compound. Lipophilic, electronic and steric parameters of the substituents are taken into account. The compounds **12h-12o** (**Figure 20**) containing the aromatic substituents proposed by TOS were synthesized as described in **Scheme 1.** For this investigation the unsubstituted benzamide **12a,** is used as the new reference compound. The choice of a reference with higher affinity is necessary to obtain better accuracy for the new generation of MAG antagonists. According to the TOS the next compound to be synthesized was *p*-Cl-benzamide **12h**. This was found to be more active than the compound **12a** (rIP=1 Vs. rIP=1.84). All other derivatives (see **figure 20**) showed lower affinity compared to **12h**. These bioassay data indicated that other electron-withdrawing substituents than chloride were not tolerated, probably because of electronic and steric reasons.

With this study, we could show that substituted mono-sialosides were better ligands for MAG than the natural oligosaccharides. A strong enhancement in binding potency was achieved by a systematic design. Specifically, starting from trisaccharide **A,** we discovered compound **12h** that exhibits a 1300-fold improved affinity. Furthermore, these compounds (e.g. **12h)** have improved pharmacokinetic properties due to the presence of aromatic moieties, lower molecular weight and a reduced number of hydrogen bond donors and acceptors.

or reductive processes.

Rostovtsev *et* al.[143] reported a very safe and efficient stereoselective synthesis of *anti*-1,4-disubstiutted triazoles through 'click chemistry'. We decided to use this reported procedure for the identification of MAG antagonists with improved affinity. The synthesis of several compounds with substituted-triazoles at the 9-position of sialic acid is outlined in **Scheme 2.** The biological data are summarized in **Table 9**.
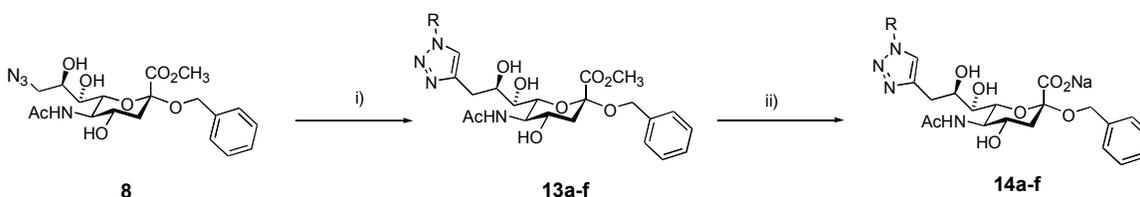
Scheme 2: i) Na-ascorbate, CuSO$_4$·5H$_2$O, tertBuOH:H$_2$O (1:1), r.t. 32%-81%. ii) a) 10% NaOH, r.t 3 hr, b) 10% HCl, Dowex 50X8 (Na$^+$), 73%-99%.

For all triazole derivatives, dissppointingly low affinities were found in the hapten inhibition assay. Nonetheless, these results further confirmed the importance of hydrogen bond donor in 9-position of sialic acid for the formation of a hydrogen bond with the MAG.

Table 9: 9-triazole substituted sialic acid derivatives.
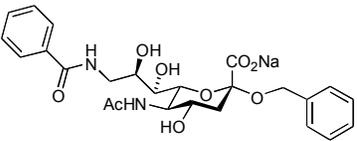
No.	Compound	rIP
12a		1.00
14a		0.03
14b		0.0008
14c		0.0007
14d		0.012
14e		0.06
14f		0.29
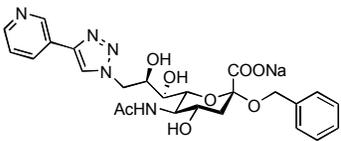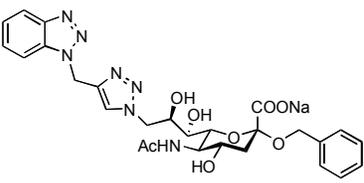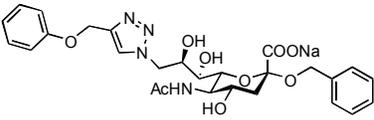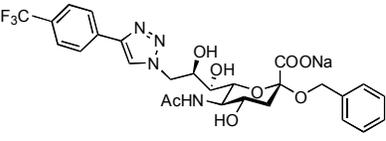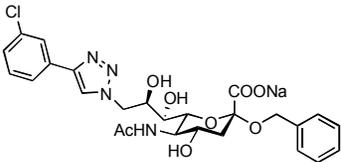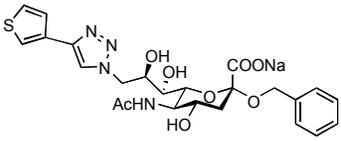
7. Biosensor Analysis:

(The Biacore studies were performed by **Daniel Strasser** from Institute of Molecular Pharmacy, University of Basel)

For the development of new pharmaceuticals, the elucidation of the dynamics of the ligand/protein interaction is of fundamental interest. Surface plasmon resonance biosensor technology provides the possibility to measure ligand/protein interactions without labeling ligand or protein and in real-time.[144] The kinetic parameters obtained are the association and dissociation rate constants (k_{on}, k_{off}), which enables a closer insight into the binding process. Since kinetic data have been shown to be useful when selecting a lead structure[145] or correlating a pharmacological effect to interaction data,[146-148] the application of this kinetic information to lead optimization will sustainably influence the drug discovery process.[147,145]

In addition to the rIPs measured by the hapten inhibition assay, the equilibrium binding constants for the compounds **12a**, **12h**, **12k**, **21** and **A** were determined by surface plasmon resonance experiments. For all ligands, the experimental data was best fitted to a 1:1 binding model. The obtained K_D values were in very good agreement with the relative affinity values (**Table 10**). In particular, the ligand with the highest rIP, compound **12h,** had the best K_D (K_D=11.8 μM). The equilibrium binding constants enabled a more detailed understanding and discussion of the results obtained in the STD-NMR and molecular modeling studies by elucidating the exact quantitative contributions of structural features for binding affinity.

For all ligand-MAG interactions, fast association and dissociation was observed (**Table 11)**. The half-life times ($t_{1/2}$) of the complexes were in the range of seconds. The obtained kinetics were in good agreement with documented kinetic properties for other carbohydrate-protein interactions.

Table 10: Comparision of equilibrium binding constants obtained by biosensor analysis together with the corresponding rIP value.

Compound	K_D +/- SEM (µM)	n	rIP
12h	11.8 +/- 1.3	3	1074
12a	20.3 +/- 3.1	3	690
12f	749.3	1	8.62
B	32.6 +/- 3.3	3	203
A	131.5 +/-	3	1

Table 11: Kinetic data obtained for the selected MAG ligands. Fast association and dissociation of the complex was observed for all measured compounds as discussed

Compound	kon +/- SEM ($M^{-1} \bullet s^{-1}$)	koff +/- SEM (s^{-1})	$t_{1/2}$ (s)	n
12h	26744 +/- 3571	0.3292 +/- 0.0447	2.1	3
12a	29916 +/-	0.5591 +/-	1.2	3
B	30589 +/-	1.0253 +/-	0.7	3
A	67264 +/-	9.1845 +/-	0.1	3

For the further improvement of binding affinities, the prolongation of the dissociation time is crucial. Unfortunately, the correlation between structural features and the dissociation rate constant K_{off} has not yet been properly defined yet. Nevertheless, using kinetic data, it would be possible to improve the lead optimization process by choosing those modifications showing slower

dissociation rather than faster association. For the MAG-project, we planned to improve lipophilic interactions starting from **12h** and to identify the corresponding contributions to K_{on} and K_{off}.

8. STD NMR:

(were performed by **Dr. Brian Cutting** from Institute of Molecular Pharmacy, University of Basel)

The difference between two NMR spectra of a ligand/receptor mixture, where in one spectrum the protein is selectively saturated, is referred to as Saturation Transfer Difference (STD) NMR spectroscopy. [149] The STD experiment provides a tool to screen compound libraries for binding to the protein as well as to map the binding epitopes of the ligand.

The protein is saturated in the presence of the ligand by the application of selective irradiation through a train of weak radio-frequency pulses, which do not directly affect the resonances of ligand. The magnetization is spread over the entire protein by intramolecular Overhauser processes and spin-diffusion, which is more efficient with larger proteins. Ligands interacting with the saturated protein are distinguished from non-binding compounds based on intermolecular Overhauser processes. Ligands which had received magnetization from the protein dissociate back into solution where they are detected.[149] Short half-lives, with respect to longitudinal relaxation, of the ligand-protein complex result in a higher sensitivity compared to monitoring the protein. This sensitivity improvement is due to the rapid mixing of the bound and free forms and extended memory due to slow relaxation. The STD experiment allows the identification of the binding epitope of a respective ligand since the size of the STD-signal depends on the intensity of the local ligand/protein interactions.

The intensity of STD NMR signals is easily measured provided the binding is between 10^{-3} and 10^{-8} M, which corresponds with affinities generally found for carbohydrate/protein interactions. In contrast to methods such as SAR by NMR,

there is no limit to the size of the protein. In fact, the increase of sensitivity goes parallel to the size of the protein due to a more efficient inter- and intramolecular saturation transfer.[150]

To investigate the structural elements of the ligands interacting with MAG, three structurally related compounds (**12a**, **D** and **B**) were measured using the STD technique. The structures of the three compounds analyzed by STD-NMR with corresponding STD-values are depicted in **Figure 21**. The common core to all three antagonists consists of the sialic acid residue. Depending on the "decorations" at the reducing end, modifications of the binding mode could be determined by STD-NMR. Whereas compound **12a** contains an *O*-benzyl substituent at the reducing end, compound **D** is a *O*-methyl glycoside and **B** a trisaccharide.

The STD reference and STD spectra, respectively, for each of the three compounds analyzed by NMR are displayed in **Figure 22**. To aid in the visual comparison of the epitope, each spectrum is shown with equivalent vertical scaling of the *N*-acetate methyl resonance. Additionally, in the STD spectra, dotted lines are drawn above the *meta*-hydrogens of the benzoate present within all three compounds. The heighted lines represents the STD effect found for the *meta*-hydrogens of **12a**.

The largest, and most easily quantified STD effects for the three compounds are collected in **Table 12**. The enhancement of the resonances for the hydrogens of the benzoate were comparable in size for compounds **12a** and **D**, however noticeably reduced for compound **B**. According to **Figure 21**, it becomes evident that the aromatic substituents exhibit strong enhancements and are thus hypothesized to be crucial for binding. For compound **B**, they turned out to remarkably smaller.

59
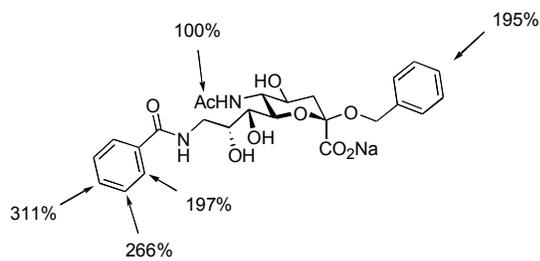
12a (K_D : 20 μM)
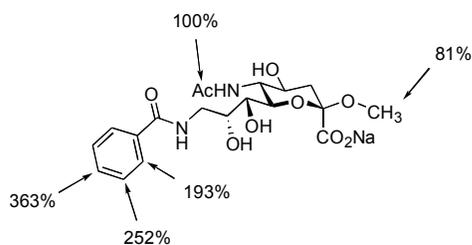
D (K_D : 131 μM)
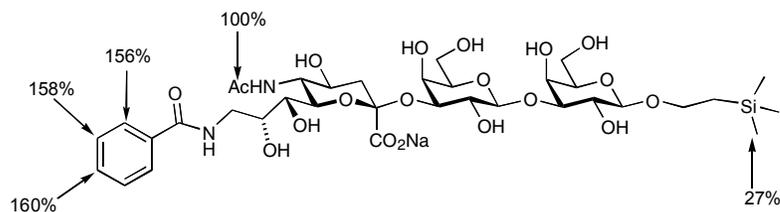
B (K_D : 33 μM)

Figure 21: Group epitope mapping of compounds **12a, D** and **B;** the figure indicates the STD-effect for the corresponding hydrogens; the STD-values are normalized to the *N*-acetate (100%).
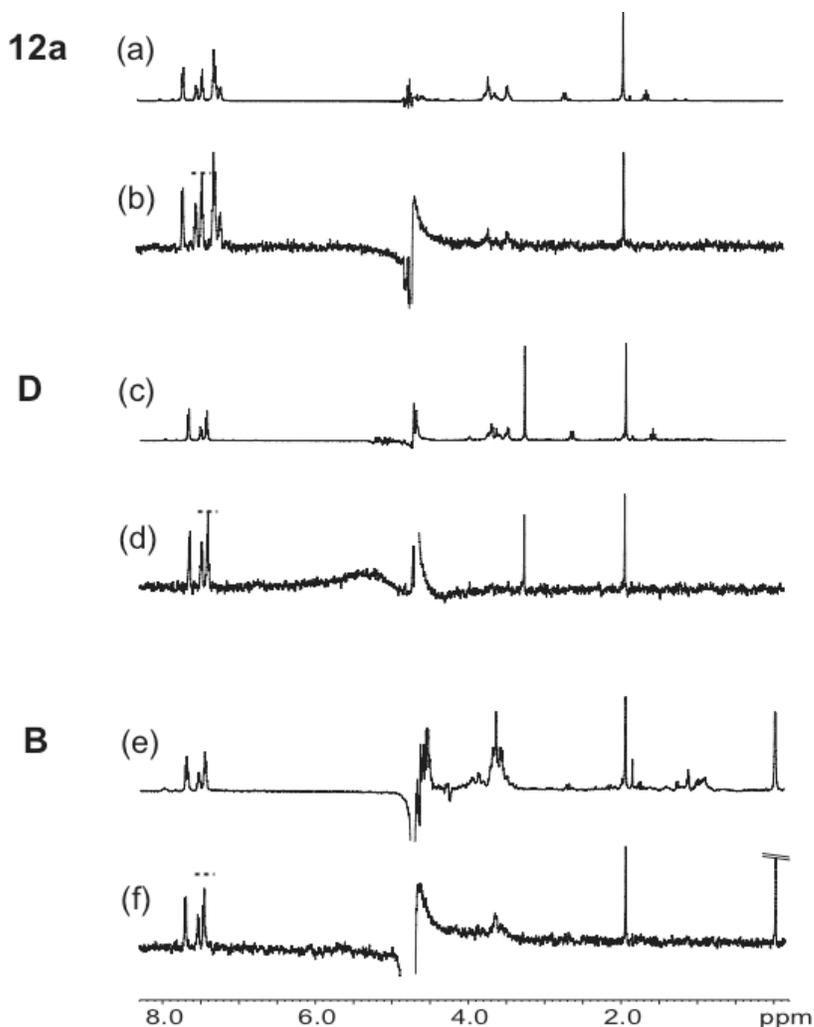
Figure 22: STD reference and STD spectra for compounds **12a** (a, b), **D** (c, d) and **B** (e, f), respectively; Each spectrum is vertically scaled to have identical intensities for the methyl group of the N-acetate moiety resonance at 1.95 ppm)

The relative error among the three types of benzamide hydrogens was largest for the *para* position since this signal is due to only one hydrogen, and consequently has a lower signal to noise ratio. Inspection of other STD enhancements, as seen in **Figure 22,** yields relatively small STD enhancements for hydrogens bound to the sugar rings.

The size of the STD enhancement of the various substituents provides insight into the origin of the affinities of different ligands determined by biosensor analysis. For compound **12a**, the benzoate experienced a strong enhancement and had the strongest binding affinity. Compound **D** displayed enhancements

similar to compound **12a** for resonances in the common core fragment, and was therefore hypothesized to have a similar binding mode.

Table 12: Summary of the STD effects for compounds **12a**, **D** and **B**.

Compound	STD H^{para}/H^{NAc}	STD H^{meta}/H^{NAc}	STD H^{ortho}/H^{NAc}	Biosensor determined K_D (μM)
12a	3.1	2.7	2.0	20
D	3.6	2.5	1.9	131
B	1.6	1.6	1.6	33

However, the replacement of a methyl group for a benzyl group reduced the affinity by a factor of 6.5. The weaker binding of compound **D** was manifested in the STD spectrum by a reduction in the enhancement factor of the methyl group by a factor greater than two. The reduced affinity of **D** and the smaller STD effect of the methyl group may be due to a smaller hydrophobic contact area of the methyl group compared to the benzyl group or a weaker binding to the receptor due to the absence of pi-stacking interactions. Interestingly, the mode of binding of compound **B** appeared different than for compounds **12a** and **D**. The change in the STD enhancement of the common fragments may be attributed to a necessary chance of the binding mode caused by steric effects of the larger disaccharide substituent. In fact, the disaccharide moiety did not show any significant STD signal. The only other side chain of **B** that received an observable STD enhancement was the TMS group. Since the affinity constant for compound **B** (K_D = 33 μM), was far closer to the stronger binding **12a** (K_D = 20 μM), than the weaker **D** (K_D = 131 μM), the observation that the TMS group received measurable STD may indicate the presence of a second potential binding region for MAG inhibitors.

9. Molecular Modelling:

(were performed by **Dr. Michele Porro** from Institute of Molecular Pharmacy, University of Basel)

To gather more information about the binding modes, in parallel with the information from the STD-NMR experiments, the most active compound, **12h**, was docked onto the MAG-model. Heiko Gaithe from University of Bremen developed the homology model of MAG from the amino acid sequence of MAG. The ligands were manually docked on to the homology model of MAG. This approach assumed a similar ligand-docking mode as that found for Methyl $\tilde{\alpha}N$-benzoyl-amino-9-deoxy-Neu5Ac in the crystal structure of Siglec-1a, protein with high homology with respect to MAGThe result is presented in **Figure 23** and **Figure 24**. The proposed docking mode showed that the carboxylic group of the ligand forms a salt bridge with Arg118. Additionally, the hydrophobic modifications at the 2-(OBn) and 9-(NHC(=O)Ph) position of the sialic acid scaffold found ideal partners for hydrophobic interactions in the sidechains of the amino-acids Phe129 and Glu131. A further hydrophobic contact between the methyl group of acetyl-moeity at position 5 and Trp22 as well as a hydrogen bond between the 5-NH-group and the backbone carbonyl group of Gln126 were observed.

The crucial hydrogen bond (see **Table 8** for affinity difference between compounds **5α**, **12a** and **C**) between the NH (donor) at the 9-position of the ligand and its protein partner was found to be the carbonyl backbone of Phe129. The docking mode presented seems to be very plausible, because of the similarity to the observed binding mode for methyl $\tilde{\alpha}N$-benzoyl-amino-9-deoxy-Neu5Ac in a homology model based on the crystal structure of Siglec-1, a protein with high homology to MAG.[84]
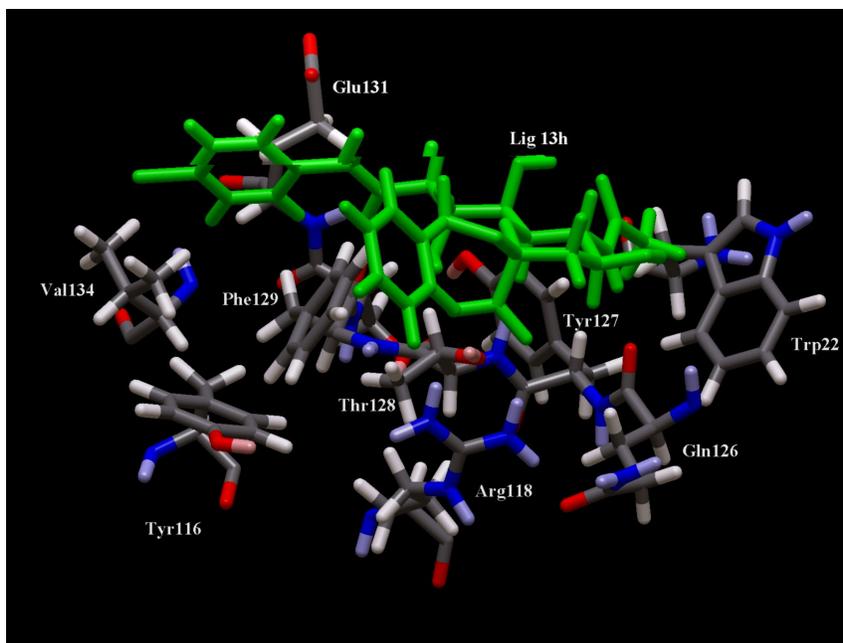
Figure 23: The docking mode obtained for ligand 12h. The ligand is presented in green.
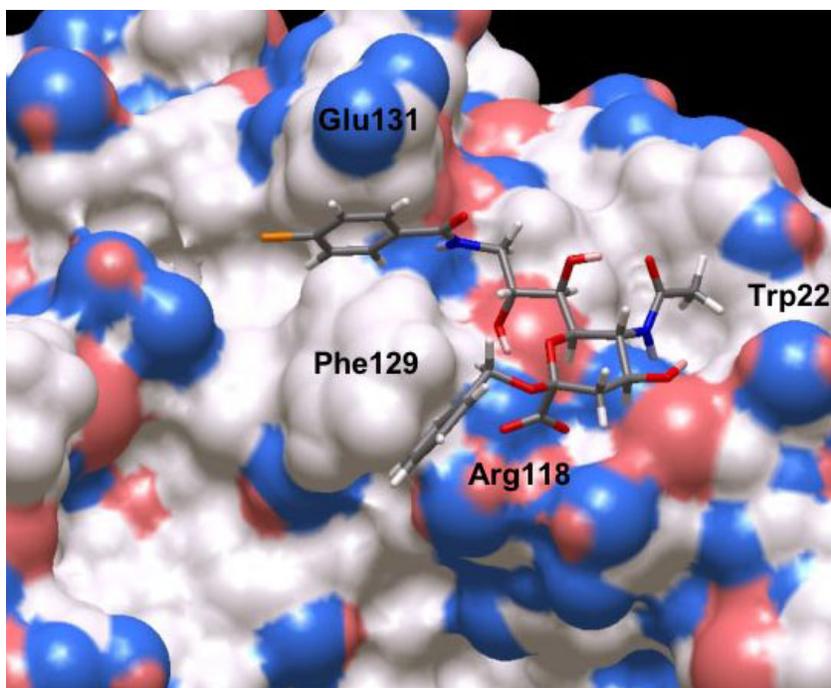
Figure 24: View of the stacking of aromatic groups of **12h** in binding pocket of MAG.

The docking mode presented is in strong agreement with the STD-NMR results. In particular, the STD signals measured by NMR that indicated strong interactions between the hydrophobic moieties of the MAG-ligands and the protein could be explained. These contacts might be rationalized as interactions between the modifications at the 9-position and the sidechains of Phe129 and

Glu131, the methyl group of the acetate at the 5-position with Trp22 and the substituents at the anomeric center with Phe129. The docking mode also explains the reduction in binding affinity observed for ligand **A** (K_D =131 μM) compared to that of ligand **12a** (K_D=20 μM). This loss in affinity seemed ideally explained by the loss of the strong interaction with Phe129, a hypothesis substantiated by a less intense signal in the STD-NMR. The proposed docking mode enabled further insight into the SAR of the different modifications at the 9-position of the ligands. It is evident that modifications such as those in ligands **12c**, **12k** and **12n** compared to our best compound **12h** are not ideally accommodated into the shallow pocket between Phe129 and Glu131.

Finally a second docking experiment with MAG was performed with the tetrasaccharide **A**. This docking mode was overlapps with the docking mode of the best ligand (**12h**).
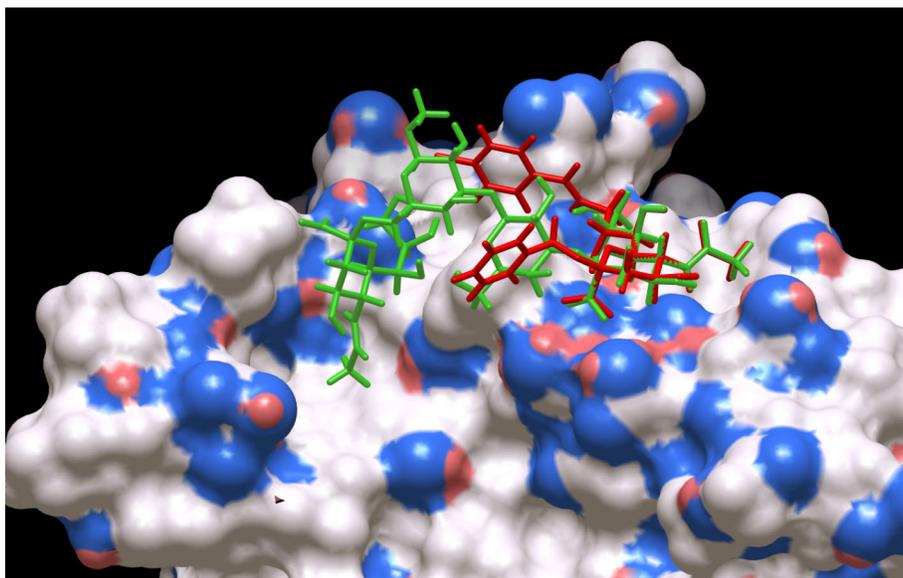
Figure 25: Overlapped docking modes of **12h (red)** and the tetrasaccharide **A (green)**

From the superimposition, it is obvious that the benzyl group at the reducing end is differently orientated than the disaccharide moiety (**Figure 25**). Based on this information, the design of a next generation of mimics is planned.

10. Pharmacokinetic evaluation of MAG Ligands

(were performed by **Dr. Michele Porro** from Institute of Molecular Pharmacy, University of Basel)

In addition to the detailed kinetic studies of the binding properties of MAG ligands by NMR, molecular modeling and Biacore analysis, profiling of the best ligand **12h,** and the tetrasaccharide **A,** was performed for the evaluation of their pharmacokinetic properties. The data is presented in **Table 13**. For good bioavailability of a compound, a molecular weight lower than 500 Da, a PSA lower than 150 Å^2, and a ClogP between zero and five are desired.

Table 13: pK properties of compounds **12h** and **A.**

12h	Tetrasaccharide A	"Rule of 5"
MW $\cong$ 537	MW $\cong$ 979	MW ≤500
PSA $\cong$ 175 Å	PSA $\cong$ 466 Å	LogP ≤5
ClogP = 1.47	ClogP = -9.752	HBD ≤5
HBA$_{Lipinksi}$ = 12	HBA$_{Lipinksi}$ = 29	HBA ≤10
HBD$_{Lipinksi}$ = 5	HBD$_{Lipinksi}$ = 16	

From the above pK data, it can be seen that the physicochemical properties of tetrasaccharide **A** is far from drug-like, since it is expected to have an extremely short half lives due to fast renal excretion. In contrast, improved drug-like molecular properties were achieved with **12h** which approaches the criteria defined by Lipinski's "Rule of 5".

11. NMR based screening

NMR spectroscopy has long been used to detect the binding of small molecules to biomolecular targets, and has more recently been employed to screen libraries of compounds for drug discovery. A variety of well known NMR techniques have been used for screening, including the nuclear Overhauser effect (NOE),[151-155]

chemical shift perturbation,[156] diffusion,[157-159] relaxation[160] and saturation transfer.[149,161,162] NMR screening can be used to detect binders with affinities ranging from nanomolar to millimolar, and recent method improvements have drastically reduced the protein consumption and increased the throughput.

11.1. Fragment based screening

An important aspect of NMR screening is the discovery of fragments that bind in proximity of a first binding site ligand, thereby offering the possibility to combine them with a short linker causing only a minimal loss in entropy. Provided a structure of the protein, and its assigned HSQC spectrum, these fragments can be found using chemical shift mapping of affected chemical shifts.[156,163] There are several general strategies by which information about weakly binding molecular fragments can be used to construct more potent molecules. One is a fragment-linking approach, in which an attempt is made to link molecules that individually bind to the target (**Figure 26**). In a pioneering technique termed "SAR by NMR",[156] NMR screening[152,155,160,164] is used to identify a ligand binding to the target through the use of an isotopically enriched protein and structural information. At saturating concentrations of this first ligand, a second round of NMR screening is then used to discover a second ligand that binds to the target simultaneously and in the vicinity to the first ligand ("second-site screening"). The fragments that bind in proximity are identified using chemical shift perturbation, and the ligands of the two binding site are linked with various length of linkers.
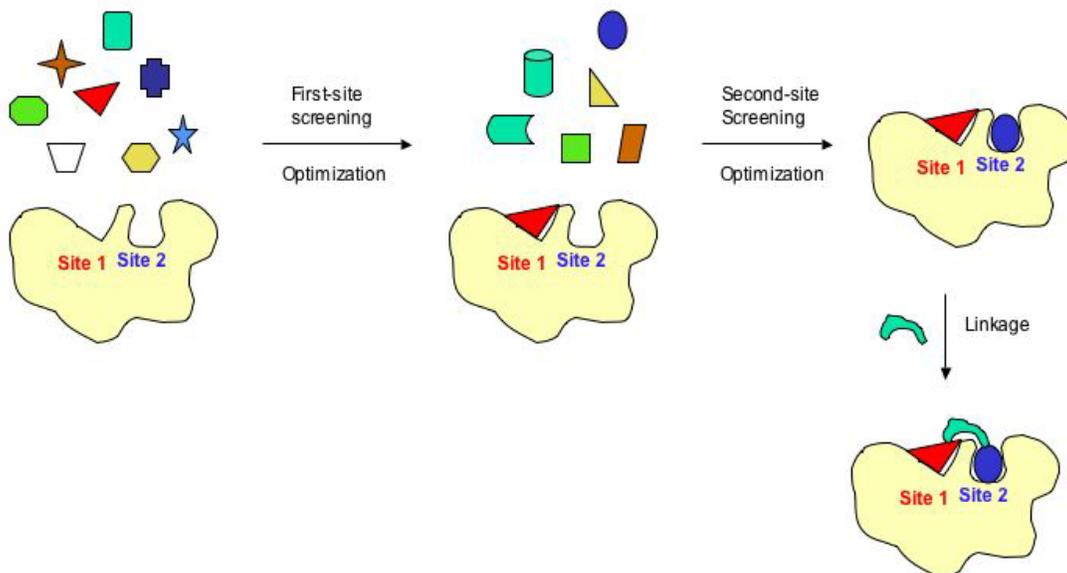
Figure 26: Fragment-Based Screening**.**

As a ligand for the first binding site in MAG was identified (compound **12h**) which showed a decent affinity (K_D=20 µM) for MAG, a second site screening approach was used to identify a proximate second binding site and ligands thereof by using the spin label advocated by Jahnke.[163]

11.2. Second-Binding Site Screening

Jahnke *et* al.[163] described an elegant method of second site screening through the use of a spin labeled first ligand. This method exploits transverse relaxation to detect ligand binding, thus the variation between the bound and free state relaxation rates of the second site ligand should be as large as possible. Since the gyromagnetic ratio of an unpaired electron is a factor of 658 times larger than that of a proton, transverse relaxation effects on a second ligand, binding simultaneously and in the vicinity of the spin labeled first ligand, will be significant.[165]

All the second site ligands will exhibit quicker relaxation due to binding to the protein. In addition, if the second site ligand binds in the vicinity of the unpaired

electron (radius of $\approx$ 20Å), its relaxation will as well be enhanced from paramagnetic interactions. This potential contribution from the later effect is distinguishable from the former by reducing the spin label and therefore knowing its effect.

11.2.1.   Second site NMR screening with a spin-labeled first ligand

After identifying a first site ligand, for MAG and gathering information regarding the binding modes (e.g. **12h**, **A**, **B**, **D**) by STD NMR and molecular modeling, we further decided to investigate the possibility of a nearby second binding site to further enhance the activity.

As shown in our previous report,[166] the tetrasaccharide core in GQ1bα, Neu5Ac-α(2→3)-Gal-β(1→3)-[Neu5Ac-α(2→6)]-Gal$N$Ac, with two terminal sialic acid moeities is an important epitope for binding to MAG. The terminal α-2,3-linked sialic acid is the more important pharmacophore. In contrast to the important contribution of the two sialic acids to binding, several SAR studies have indicated that the disaccharide core Gal-β(1→3)-Gal$N$Ac, acts predominantly as a linker holding the two sialic acid moieties in the appropriate spatial orientation.[57,77] This hypothesis was based on in-house STD-NMR studies, from which only small STD effects the disaccharide core were observed.

The spin-labeled derivative **12a*** which is based on the ligand **12a** for the first binding site was synthesized. The rIP=0.15 and rIC$_{50}$=2.98 values of the spin-labeled derivative **12a* (Figure 27),** clearly shows that the activity was not considerably reduced compared to the reference compound **12a,** suggesting a similar mode of binding. This result also demonstrates that the addition of the TEMPO moiety did not significantly disturb the binding, thus allowing compound **12a*** to be used for screening second site ligands.
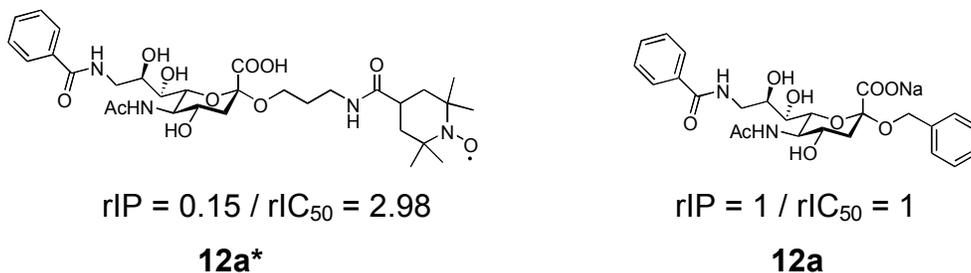
Figure 27: Compounds 12a and 12a*
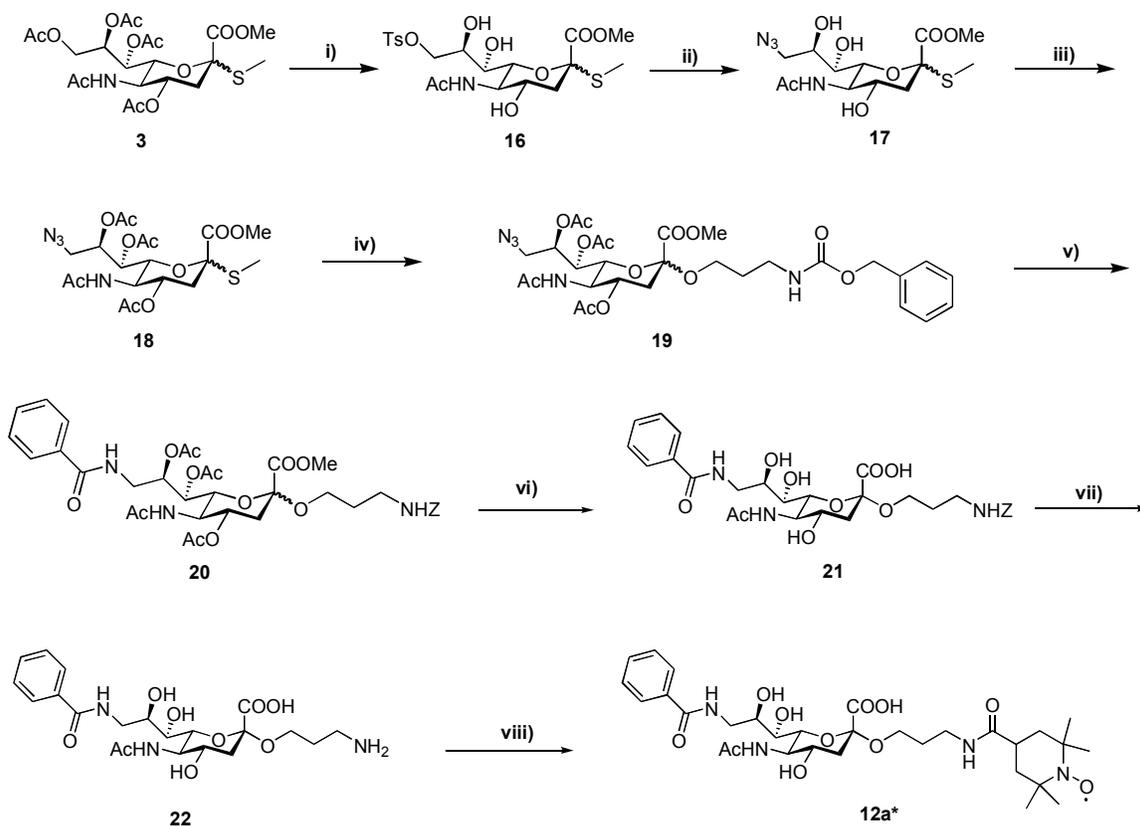
Scheme 3: i) 1 M NaOMe, MeOH, 2 h, Amberlyst H+, 68%; vi) p-TsCl, pyr, 56%; vii) NaN₃, DMF, 18-C-6, 89%; viii) Ac₂O, DMAP, pyr, 90%; vi) NIS, TfOH, Cbz-amino propanol, ACN, -40°C to – 30°C, 14 h, v) PhCOCl, PPh₃, DCE, r.t. 14 h, 95%. vi) a) 1 M NaOMe, MeOH, r.t. 2 h. b) H₂O, 2 h, 52%. vii) [H]₂, Pd/C, MeOH, 2 h, 95%. viii) a) Dhbt, DIC, THF, O°C, 2 h. b) 4-carboxy-TEMPO, DMF, r.t. 2 h, 57%.

The synthesis of compound **12a*** (**scheme 3**) was started from a building block

18. Compound **3** was deprotected (→**15**) and selectively *p*-tosylated under standard conditions to give **16**. Introducing azide (→**17)** and further acetylation afforded compound **18**. Sialidation of Cbz-amino propanol with **18** under standard NIS/TfOH conditions gave an anomeric mixture of compound **19** (α:β 1:0.4) together with approximately 10% of glycal. The benzamide group was introduced by modified Staudinger conditions[131] to get **20** with 95 % yield. Further selective deprotection under the Zemplen conditions afforded after chromatographic purification with α-isomer **21** in 52%. The β-isomer and glycal were isolated as impurities. Hydrogenation of compound **21** in methanol gave compound **22** in 95% yield.

The next step towards the synthesis of spin-labeled compound was the coupling of compound **22** with the spin-label. 1-Oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine (**23**) was activated with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) in the presence of diisopropyl carbodiimide (DIC) and the intermediate **23a** (**Scheme 4**) was condensed with the free amine of compound **22** according to the reported procedure.[167] The final product was purified by reversed phase chromatography which yeilded compound **12a*** in 52% yield.
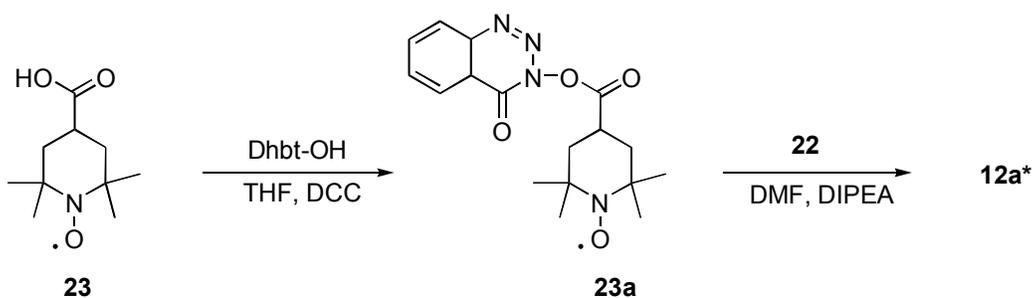
Scheme 4

11.3. Library Design for NMR screening

The next step towards finding a second site ligand was to design an appropriate library for NMR screening. In an academic enviroment, the size of the library to

be screened has to be smaller than in large pharmaceutical companies, indicating need to compose small libraries with optimizes components. Four principal criteria for library design were taken into account: diversity, drug-like character, solubility and synthetic accessibility.

A popular approach for designing screening libraries is to bias the selection of compounds towards drug-like molecules. The library is built from components, which possess desirable properties of the drugs such as low toxicity, high oral absorption and permeability, resistance to metabolic degradation and the absence of rapid excretion. In contrast to other methods for compound classification, an appealing approach is to construct a library around molecular scaffolds that occur frequently in known drugs.[155]

Despite the emphasis on drug-like molecules, the goal of primary screening is to produce good leads, not drugs. As described by Teague and coworkers,[168] it is no sufficient that drug-like leads fulfill Lipinski's 'Rule of 5'. Such leads are not an optimal starting points for optimization because the addition of lipophilic groups that increase potency can MW and logP. Therefore drug-like leads are smaller (MW=100–350 Da) and more polar (clogP 1–3).[168] The implication is that libraries biased towards small, polar molecules are inherently superior. This is advantageous for NMR screening, which benefits from both high solubility and relatively weak binding.

Properties used to select drug-like compounds:

<u>Rule of 5' criteria</u>[169]

• Molecular weight ≤500 Da

• LogP ≤5

• Hydrogen bond donors (OH/NH) ≤5

• Hydrogen bond acceptors (O/N) ≤10

<u>Other criteria</u>[170-172]

• Number of heavy atoms 10–70

• Rotatable bonds 2–8

• Number of rings 1–6, aromatic ≤3

• Molar refractivity 40–130

A complementory advantage of small, simple molecules are that they can penetrate deep into active sites without steric hindrance. Of particular interest in

academic settings is that, the number of compounds necessary to represent the scaffolds of interest is relatively small. Taking the above literature findings, the SHAPES strategy was applied to identify the molecules to be compiled in the library to identify second site hits by NMR.

11.3.1. The SHAPES strategy:

Design of the SHAPES library is based to a large extent on previously published work, in which commercially available therapeutics were examined for common chemical features that made these compounds 'drug-like'.[173] In this study, all drug molecules were broken down into systems consisting of rings, linkers and side chains. The library is composed largely of molecular shapes that represent frameworks most commonly found in known drugs. [173] A set of frameworks from which the 'SHAPES' Library was derived is shown in **figure 28.**

For NMR screening, the compounds used in the library also must yield a simple, well-resolved 1H NMR spectrum. After a thorough literature search on library design, common occurring scaffolds in drugs were identified and were ordered from commercial sources.
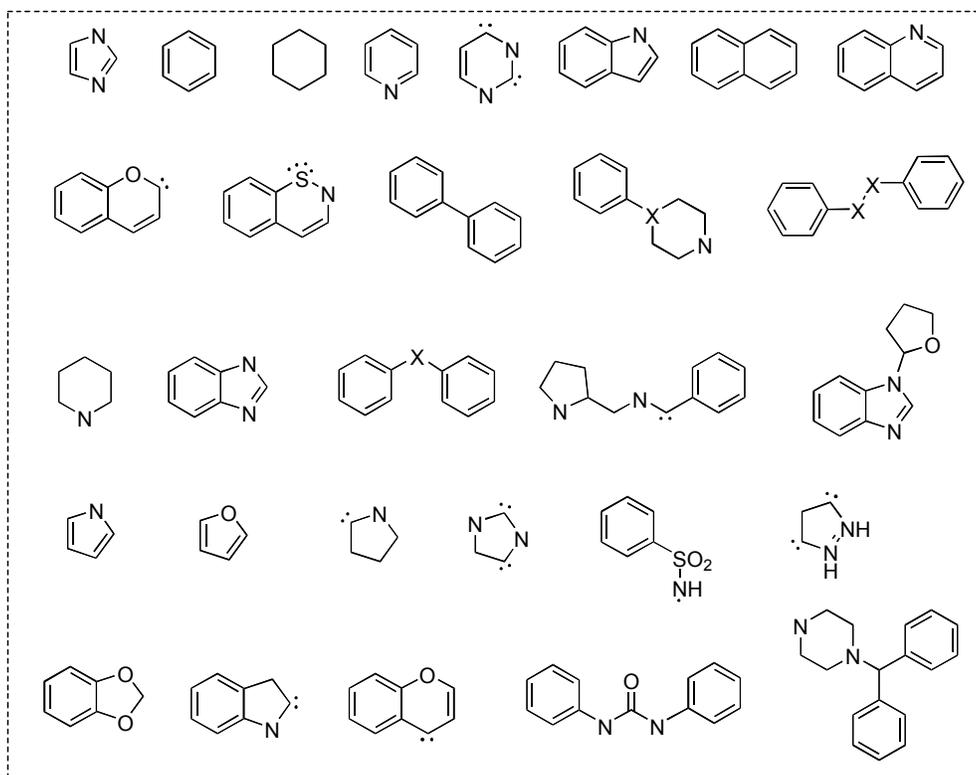
Figure 28: Molecular frameworks used for library selection. Attachment points forside chains are indicated by single electrons or lone pairs. 'X' represents C, N, O or S atom.

A small library of approximately 40 compounds (**Figure 29**) was identified and used for NMR screening. The library was divided into mixtures containing ≈ 6-8 compounds each. The mixtures were designed to minimize the intermolecular reactions and to show at least some separation of peaks on the spectra to allow each compound to be identified.
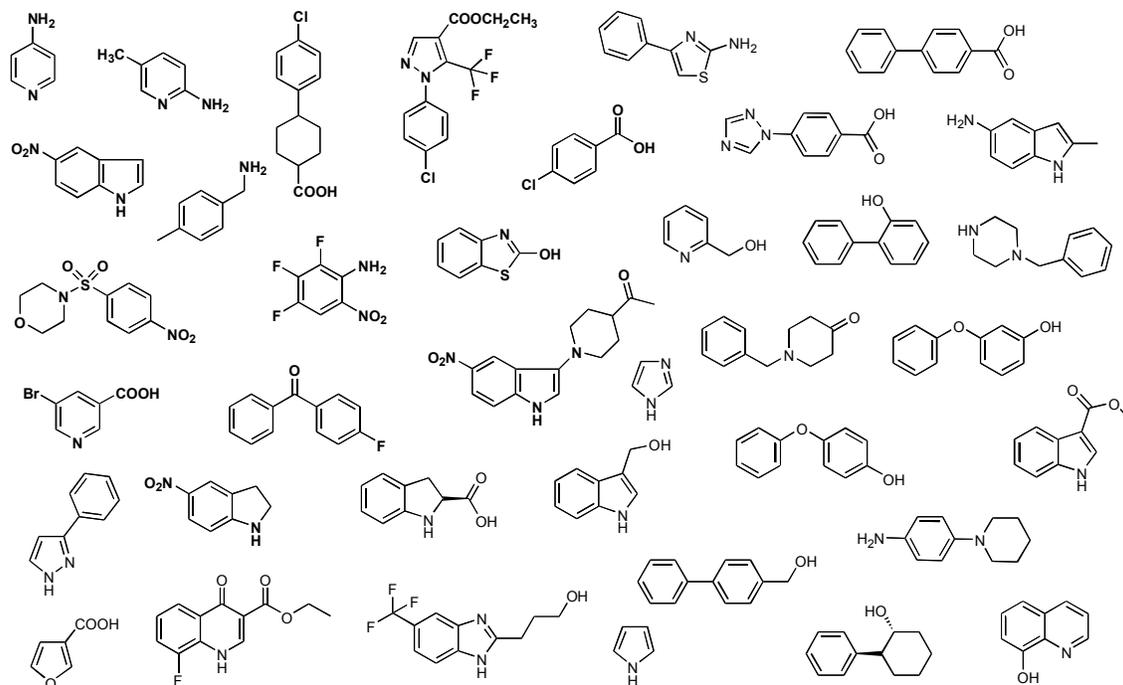
Figure 29: A library of compounds for NMR screening**.**

11.4. Library Screening

(were performed by **Dr. Brian Cutting** from the Institute of Molecular Pharmacy, University Of Basel)

After the synthesis of the TEMPO derivative of sialic acid (**12a***) and the optimization of second site library, the next step was the identification of second site ligands. The first step in the screening process was to cross validate the binding of **12a*** and the conditions for its reduction. The hapten inhibition assay confirmed that the binding affinities of **12a*** and **12a** (section 11.2.1) are not significantly different. The binding of **12a*** observed in the hapten inhibition assay was also observed with NMR as depicted in **Figure 30**-C.
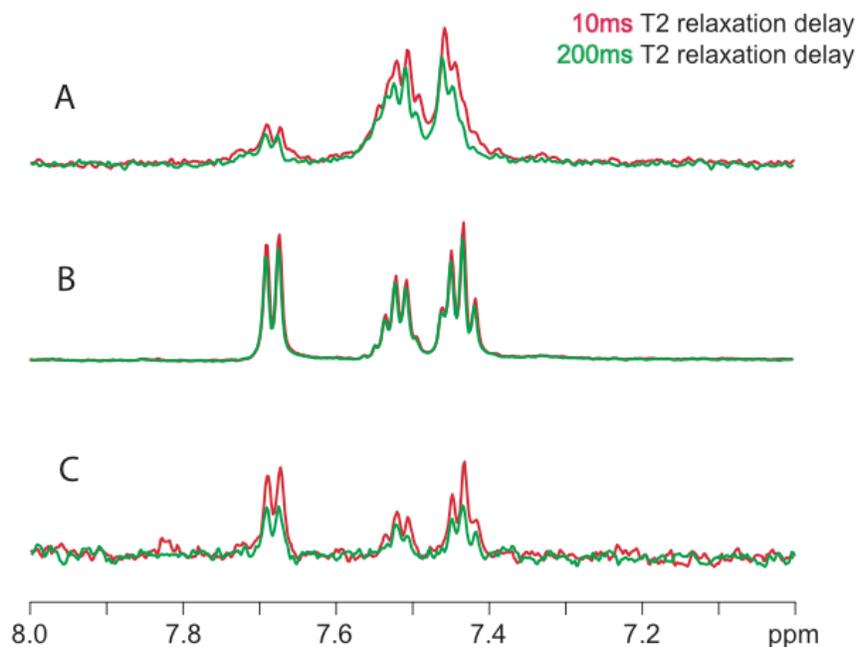
Figure 30: Reduction and hit validation of compound **12a*** for occupying first binding site**.** A) 0.5 mM spin-label **12a***. B) 0.5 mM spin-label **12a***, 16 mM ascorbic acid. C) 30 µM compound **12a***, 1 mM ascorbic acid and 3 µM MAG protein.

To validate the paramagnetic activity and conditions for the reduction, several spectra were required. First, two spectra were recorded with 0.5 mM of spin-label **12a*** in D_2O using a Shigemi tube. The spectra were recorded at T2 relaxation delay of 10 ms and 200 ms to verify the large decay in intensity due to paramagnetic relaxation (**Figure 30**-A). These two experiments were repeated after reducing the radical with 16 mM solution of ascorbic acid. The excess of ascorbic acid was used to ensure a complete reduction of the radical. The line narrowing and reduction of signal decay for the 200 ms decay proves that **12a*** is in its diamagnetic state (**Figure 30**-B). To verify the binding of reduced **12a***, 3 µM of MAG was mixed with 30 µM of **12a*** and 300 µM of ascorbic acid. As seen in **Figure 30**-C there is a larger signal decay after 200 ms than in the absence of MAG (**Figure 30**-C), thus confirming the binding of **12a*** to MAG.

After optimizing NMR conditions for observing the binding of compound **12a*** to MAG, the next step was to screen various mixture of ligands to identify second

binding site ligands. For second site NMR spectroscopic screening, MAG and spin-labeled ligand **12a*** were mixed with various sublibraries. Binding to a second site is observed by an enhancement in the transverse relaxation rate of the compounds in the library. The enhanced relaxation is potentially due to two sources. Any compound in the library, which binds to MAG will experience quicker relaxation due to the longer rotational correlation time of the bound complex. In addition, if the second binding site is in the vicinity (r ≈ 20Å) of the unpaired electron in **12a***, additional relaxation enhancement is due to paramagnetic relaxation. Paramagnetic relaxation enhancement effects from **12a*** to any of the compounds were investigated by T1ρ relaxation experiments.[163] The proximity of the simultaneously binding compounds was evaluated by analyzing the same mixture with a sufficient amount of ascorbic acid to ensure that **12a*** is in its completely reduced form. Among the various mixtures screened, nine compounds were found to bind to MAG.

From these screening hits, three compounds with indole scaffolds (**Figure 31**) were found to bind in close vicinity of first binding site. The other six compounds were found to bind too far from **12a*** since they did not show a reduced rate of relaxation upon addition of ascorbic acid. The most promising second site hit identified was found to be 5-nitro-1H-indole (marked in circle below).

Hits for second binding site
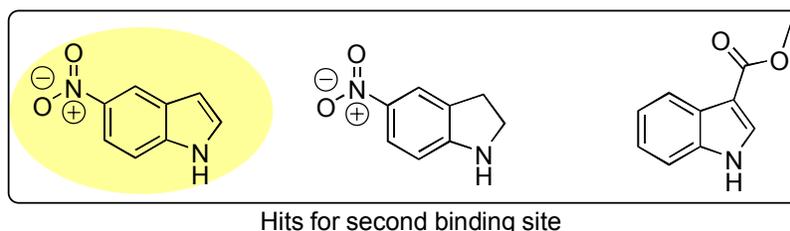
Figure 31: Identified Hits from NMR screening.

Figure 32 shows the resonances of the most promising hit (5-nitro-1H-indole) that binds in close proximity to the first binding site. As seen in **Figure 32**, a comparison of the spectra of a sublibrary shows that most compounds did not experience relaxation enhancement. However, the resonances of one compound, highlighted within dotted boxes, are completely quenched on binding

in the presence of the oxidized form of **12a***. This compound was judged to be in close vicinity of **12a***, since the peaks recovered when the spin label was reduced with ascorbic acid. This compound was therefore unambiguously identified as a second-site ligand.

In a typical experimental procedure for screening second site ligands, a spectrum is recorded for a sublibrary containing 500μM of each of the 6 to 8 ligands with short and long relaxation decay (10 ms and 200 ms). These two experiments are repeated with a second sample containing 6 μM of MAG, 180 μM of spin-label **12a*** and the sublibrary containing at 60μM for each ligand to a Shigemi 5 mm NMR tube. Finally, the two experiments were again repeated after the addition of 1,800 μM ascorbic acid to ensure the complete reduction of the radical and estimate the proximity of the two binding ligands.

In order to more confidently observe the effects of paramagnetic relaxation on the hit identified (**Figure 31)**, the ratio of ligand to protein was increased. Increasing the ratio from 10:1 to 20:1 (ligand:protein) prevented the resonances of the test compound from being completely quenched on binding by decreasing the molar fraction of bound ligand (**Figure 33**). The binding of the best hit identified was confirmed through a separate analysis, in which the non-binding ligands were not added to the Shigemi tube, by T1ρ as well as STD experiments. As shown in **Figure 33** and **Figure 34**, the identified screening hit binds simultaneously in close proximity to first binding site and thus is a confirmed second binding site ligand.

red = 10 ms relaxation delay
green = 200 ms relaxation delay
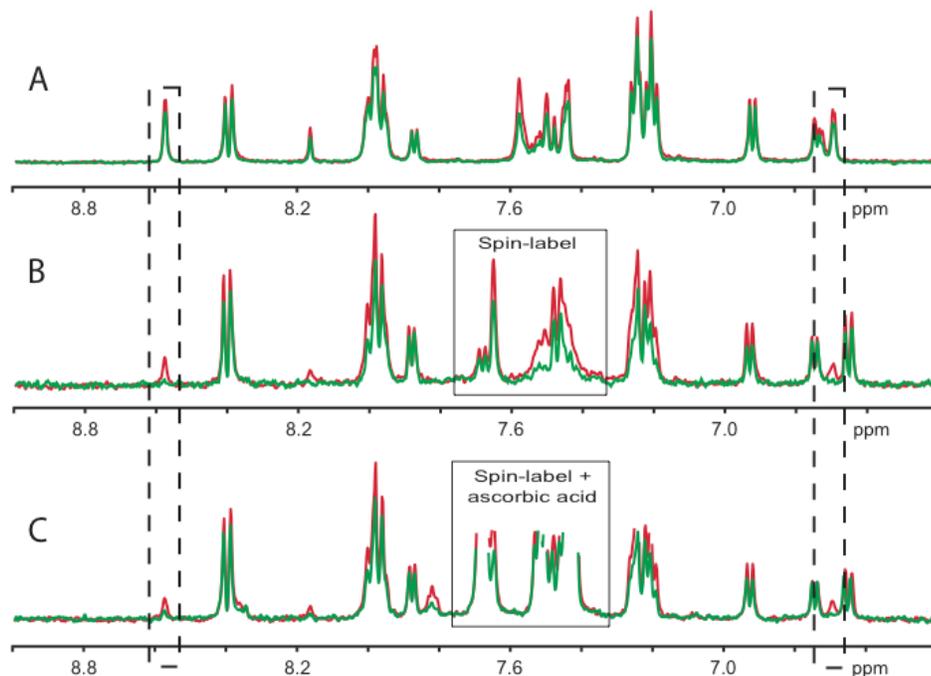
Figure 32: **Mixture analysis showing the best hit identified by T1ρ experiments.** A) A mixture of 8 compounds (500μM each). B) MAG (6μM in binding site), spin-label **12a*** (180μM), a mixture of 8 compounds (60μM each). C) MAG (6μM in binding site), spin-label **12a*** (180μM), a mixture of 8 compounds (60μM each) and ascorbic acid (1,800μM).

STD measurements were used as a complementary approach to verify the simultaneous and proximate binding to MAG of the screening hit and **12a***. The STD of the screening hit binding to MAG was measured without (**Figure 34**-A) and in the presence of **12a***. In the presence of **12a***, the STD intensities of the screening hit are measurably reduced (**Figure 34**-B). The STD signal reduction could arise from several sources, such as a modification of the K_D of the screening hit caused by structural changes in MAG or an increase in the relaxation rate of the screening hit due to the paramagnetic influence of **12a***. To evaluate if the reduced STD intensities are indeed due to paramagnetic relaxation, a third STD was measured with reduced **12a*** (**Figure 34**-C).

Figure 33: Individual analysis of identified hit by T1rho method. A) Mixture of 8 compounds at 500μM each. B) MAG (6uM in binding site), spin-label (180μM) and hit (120μM). C) MAG (6μM in binding site), spin-label (180μM), hit (120μM) and ascorbic acid (1800μM).

The STD intensities of the screening hit and **12a*** in its reduced form in the presence of MAG showed a value between its STD measured with **12a*** in its oxidized form and its STD measured in the absence of **12a***, thus substantiating the claim that the two compounds bind in close proximity to each other.
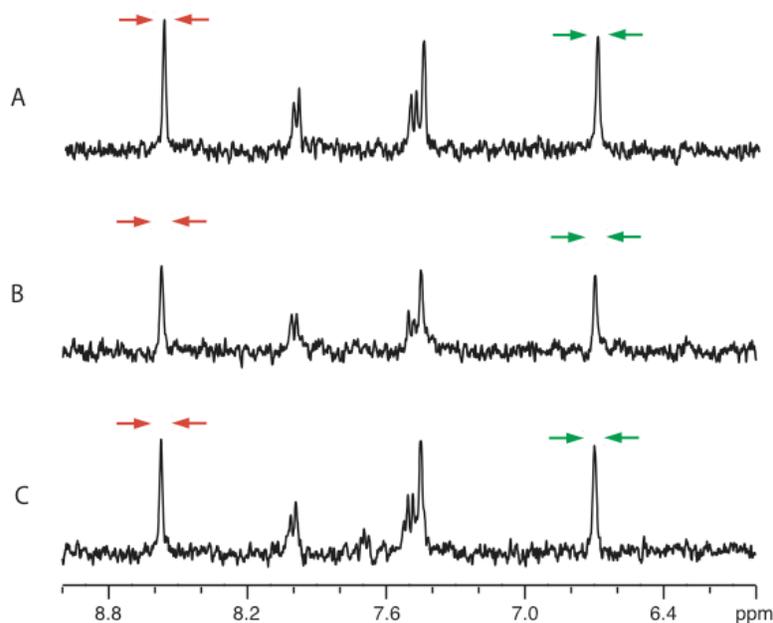
Figure 34: Individual analysis of the identified hit by STD NMR method. A) STD of second binding site hit and MAG. B) STD of second binding site hit, spin-label **12a*** (oxidized) and MAG. C) STD of second binding site hit, spin-label **12a*** (reduced) and MAG.

12. Linker design

In general, both, the first-site ligand and the second-site ligand are generally low-affinity ligands with dissociation constants in the µM or mM range. They need to be chemically linked in order to obtain a high-affinity, nanomolar ligand. The optimal linker should satisfy three criteria. First, it should allow the two individual components to occupy in the same binding site in the same orientation in the linked compound as they do as in the unlinked fragments. Second, while allowing the two components to have sufficient flexibility to adopt optimal binding orientations, the linker should be as rigid as possible in order to reduce the entropic cost of binding.[174] Thirdly, the linker should not have unfavorable interactions with the protein;

The atoms on both fragments to which the linker should be attached can be identified by observing the quenching effects of each ligands. In the case of second site screening with a spin-labeled first ligand, the paramagnetic relaxation

enhancement depends on the inverse sixth power of the distance. This mechanism leads to differential quenching effects on the second ligand, depending on its proximity to the paramagnetic center. Those resonances of the second ligand that are most strongly affected are located nearest to the first ligand and are therefore primary candidates for linker attachment.

As the crystal structure of MAG is not known till to date, we decided to identify the appropriate linker by using the *in-situ* click chemistry approach.[140-142,175-177]

13. *In-situ* click chemistry

The *in-situ* click chemistry approach to target-guided synthesis (TGS) employs the completely bioorthogonal [1,3] dipolar cycloaddition reaction between azides and acetylenes which was pioneered by Rolf Huisgen.[134] This process is self-contained, hence there are no external reagents, catalysts, or byproducts that might interfere, and the reactants themselves are "invisible" in biological milieu. Most importantly, the cycloaddition reaction, which is slow at room temperature, is accelerated tremendously when azide and acetylene groups are held together in close proximity to lead to irreversible formation of triazoles.[178-180] Sharpless *et al*.[140-142,175-177] successfully employed this approach to assemble acetylcholinesterase (AChE) and carbonic anhydrase inhibitors (CA). A femptomolar inhibitor[175] for AChE was identified by *in-situ* click chemistry approach.

13.1. Synthesis of precursors for first binding site and second binding sites

For the *in-situ* click chemistry, the azide and alkyne moieties have to be introduced into the ligands to be linked. We decided to incorporate the azide group on 5-nitro indole and the alkyne groups on the sialic acid. The alkynols

were selected with varying spacer lengths and the azide group with a varying spacer lengths on the 5-nitro-1H-indole in order to identify an optimum spacer length and orientation of ligands in respective binding site pockets.

13.1.1. Precursors for first binding site: (SA1-4)

The sialidation with various alkynols was performed as described in literature.[181] As shown in **scheme 5,** alkynol (n= 1,2,3,4) was coupled with 2-chloro-sialic acid derivative **24**[182] to give compound **25a-d** as a mixture of α and β isomers in 1:1 ratio. This mixture was O-deacetylated to give compound **26a-d** from which α and β isomers could be separated. The α-isomer of compound **26a-d** was further selectively p-tosylated at 9-position to yeild compound **27a-d**. After the introduction of an azide group ($\rightarrow$ **28a-d**) and acetylation compound **29a-d** were obtained in good yields. Amidation under the modified Staudinger reaction[131] gave compound **30a-d**. A final deprotection with 10% NaOH in methanol, neutralization with 10% HCl and finally passing it through a Dowex 50X8 (Na$^+$) afforded the Na-salts of compound **31a-d**.
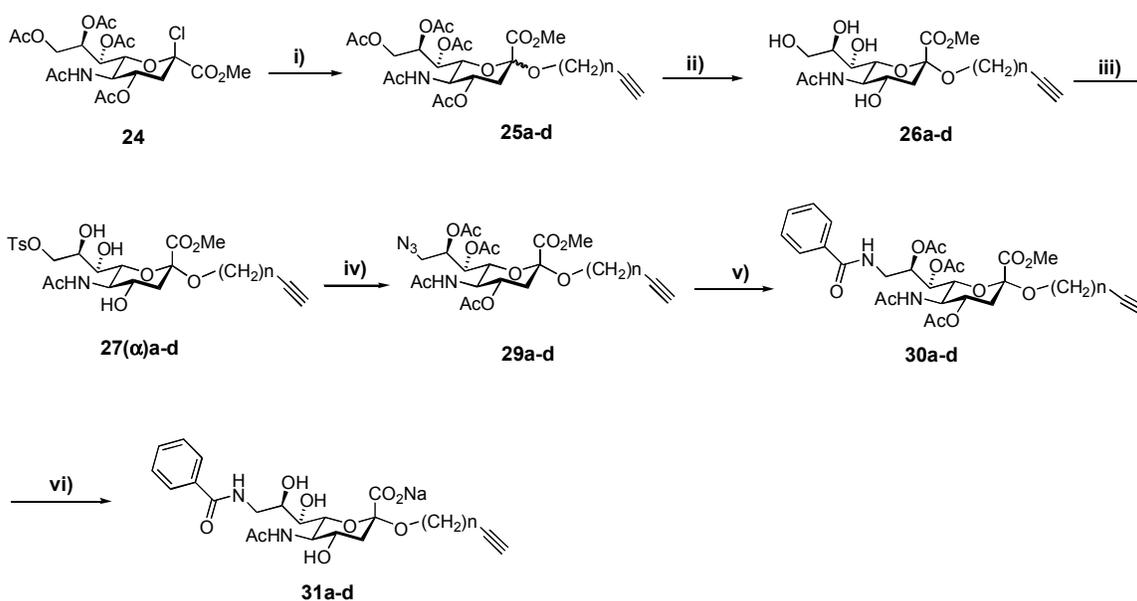
Scheme 5: i) AgOTf, CH$_3$CN, MS 3 Å, r.t. alkynol, . ii) 1M NaOMe, MeOH, Amberlyst 15 H$^+$, r.t. 2

h. iii) *p*TsCl, pyr, 0°C. iv) a. NaN$_3$, DMF, 60°C, 24 h. b. Ac$_2$O, pyr, DMAP. v) PhCOCl, PPh$_3$, DCE, r.t. 16 h. vi) 10%NaOH, MeOH, Dowex 50X8 (Na$^+$).

13.1.2.   Precursors for the second binding site: (NI1-3)

The introduction an azide group with different spacer length on 3-position of 5-nitro-1H-indole followed different synthetic routes. The 3-(azidomethyl)-5-nitro-1H-indole **39** was synthesized from commercially available 5-nitro-1H-indole **32** (**scheme 6**). **32** was formylated at the 3-position to get 5-nitro-1H-indole-3-carboxaldehyde **33**.[183] After reduction with NaBH$_4$ in methanol to 5-nitro-1H-indole-3-carbinol **34** was isolated. Due to the high reactivity of *N*H of indole, the nitrogen was protected by a *t*BOC group to yeild compound **35** with 62% yield. The other side product was the *di*-BOC derivative. By SN2 substitution compound **35** was transformed into corresponding bromide **37** (62%) with bromo-alkene **36**[184,185]. Substitution of the bromide with an azide group yeilded compound **38** in 95% yield. The deprotection of the nitrogen in TFA afforded compound **39** as a yellowish solid with 71% yield.
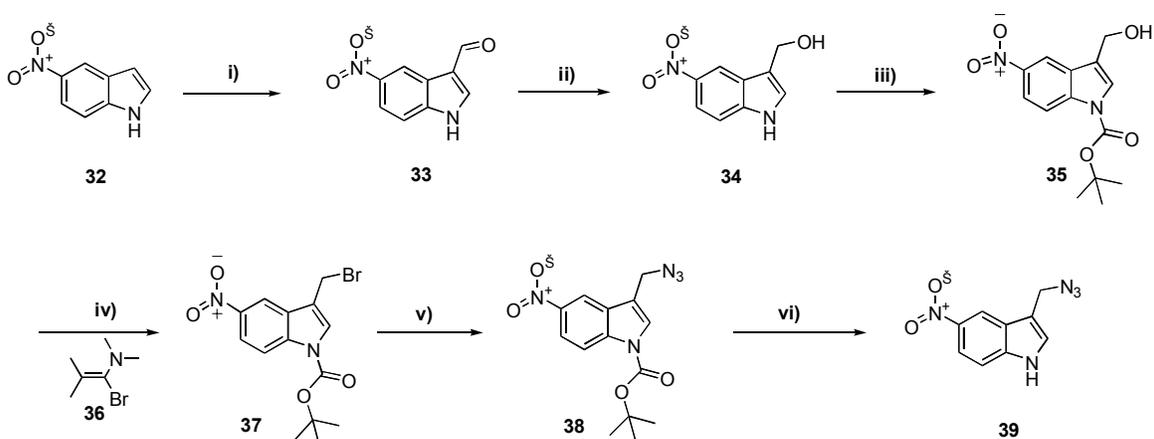
Scheme 6: i) POCl$_3$, DMF, 92%. ii) NaBH$_4$, MeOH, 93%. iii) Boc$_2$O, DMAP, DCM, 14 h, 62%. iv) 19, CHCl$_3$, r.t. 6 h, 62% v) NaN$_3$, 15-C-5, DMF, r.t. 16 h, 95%. vi) TFA, DCM, 70%.

As reported[186], 3-(3-azidopropyl)-5-nitro-1H-indole **43** was also synthesized from 5-nitro-1H-indole **32** (**scheme 7**). The 5-nitro-1H-indole **32** was treated with Meldrum's acid, formaldehyde and proline in ACN to give the compound 5-[(5-

84

nitro-1H-indol-3-yl)methyl]-2,2-dimethyl -1,3-dioxane-4,6-dione as a yellowish solid. Without further purification, the crude product was heated to reflux in ethanol in presence of pyridine and copper to give ethyl 5-nitro-3-(1H-indole)propionate **40**. Compound **40** was then reduced with LiAlH₄ in THF to give 5-nitro-3-(3-hydroxypropyl)-1H-indole **41** in 82% yield. Treatment of **41** with bromoenamine **36**[184,185] in CHCl₃ yeilded 3-(3-bromopropyl)-5-nitro-1H-indole **42** (62%). Treatment of **42** with NaN₃ in DMF gave 3-(3-azidopropyl)-5-nitro-1H-indole **43** as a yellowish solid in 96% yield.
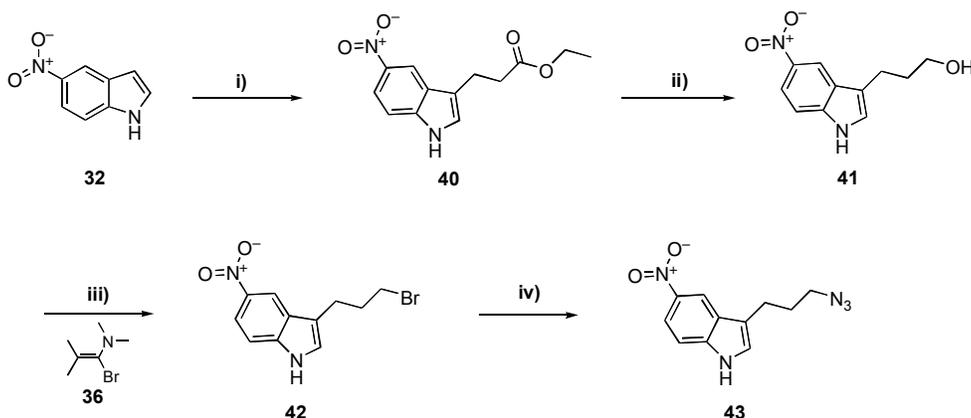
Scheme 7: i) a. MA, HCHO, proline, CH₃CN, r.t. 20 h. b. Cu, pyr, EtOH, reflux, 2 h. ii) LiAlH₄, THF, 82%. iii) **36**, CHCl₃, r.t. 6 h. iv) NaN₃, 15-C-5, DMF, r.t. 16 h, 96%.

13.2. In-situ click chemistry experiments

After the synthesis of the first and second site ligand, *in-situ* click chemistry reactions (**scheme 8**) were conducted in Eppendorff vials.
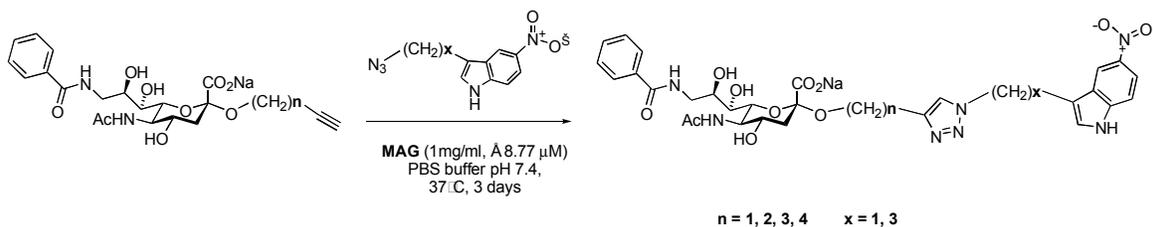
Scheme 8.

Each Eppendorff vial contained a mixture of MAG (8.77 µM), the acetylenes **31a-d** (380 µM), and the azides **39/43** (760 µM) in phosphate buffer solution (pH 7.4).

For a simple understanding of abbreviations, the acetylenes were named as **SA1-4** and azido-nitro-indoles as **NI1,3**, with the numericals indicating the number of carbon atoms in C-chain. The formation of the product was monitored by LC-MS-SIM analysis. Two sets of control experiments were conducted in parallel to check the false positives, one with a bovine serum albumin (BSA) in place of MAG and another in the absence of any protein. Each mixture was stirred at 37°C for 3 days.

13.3. Analysis of the in-situ click chemistry reactions
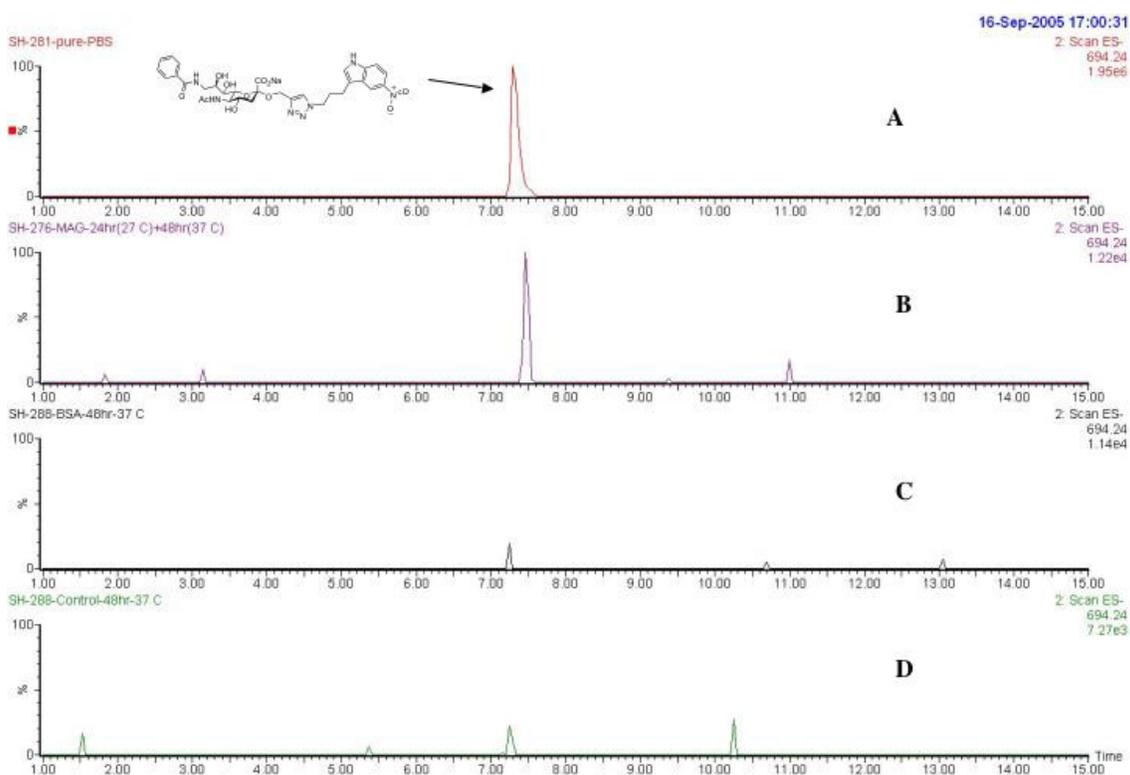
Figure 35. The *in-situ* product **SA1NI3** compared by LC-MS-SIM analysis to authenticate sample from the Cu(I)-catalysed reaction. A) Authentic sample of *anti*-**SA1NI3** synthesized by Cu(I)-catalyzed reaction. B) **SA1-4** and **NI3** incubated at 37°C at 48 hr in the presence of MAG. C) **SA1-4** and **NI3** incubated at 37°C for 48 hr in the presence of BSA. D) **SA1-4** and **NI3** incubated at 37°C for 48 hr in the absence of any protein.

The analysis of each mixture by LC-MS-SIM revealed the exclusive formation of a triazole product in the presence of MAG. The formation of this triazole product was neither observed in presence of BSA, nor in absence of protein. This observation validates the fact that the identified product is an *in-situ* hit and its formation required both binding sites to be easily accessible. **Figure 35** illustrates the results for identified **SA1NI3** combination.

For the determination of binding affinities, all 8 *anti*-triazoles were synthesized (**Scheme 9**) from their respective acetylene/azide precursors using the copper-catalyzed reaction conditions developed by Sharpless/Kolb[143] and Meldal.[187] The bioaffinites of these compounds were measured in the Biacore assay that was developed in house.

n = 1, 2, 3, 4   x = 1, 3
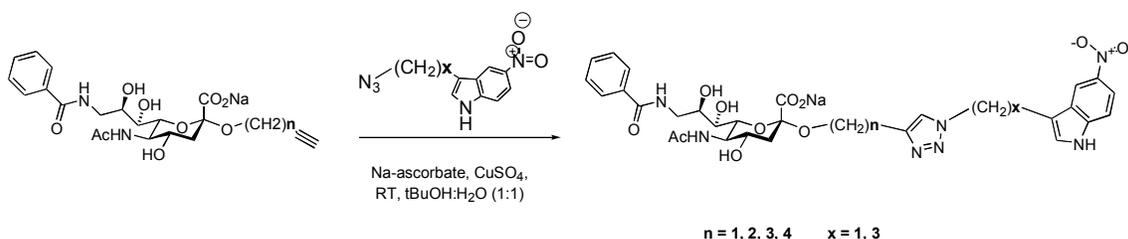
Scheme 9.

13.4. Bioassay

(Biacore assay was performed by **Daniel Strasser** from Insititute of Molecular Pharmacy, University of Basel)

As shown in **Table 14**, repeated measurements from the Biacore assay show high binding affinity for the *in-situ* hit **SA1NI3** (K_D = 190 nm). This value is significantly stronger than either the acetylene precursor **SA1** (K_D = 76.5 µM) or azide precursor **NI3** (K_D = >100 mM) from which they are derived (**Table 15**).

This shows that the *in-situ* click chemistry can be used to selectively identify the strongest binding inhibitors for MAG, in which only one of the components binds well to the target.

Table 14: K_D values for triazole-linked compounds. (NA = Not active)

n	x = 1		x = 3	
	K_D	SD	K_D	SD
1	NA	-	0.19 µM	+/- 0.01
2	NA	-	1.00 µM	+/- 0.41
3	NA	-	1.74 µM	+/- 0.31
4	NA	-	3.63 µM	+/- 0.40

As shown in **Table 15**, the **SA1-4** compounds were found to reasonably active in Biacore assay. The increase in the chain length of the spacer decreases the activity. In case of **NI1,3** it was observed that the activity of **NI3** is
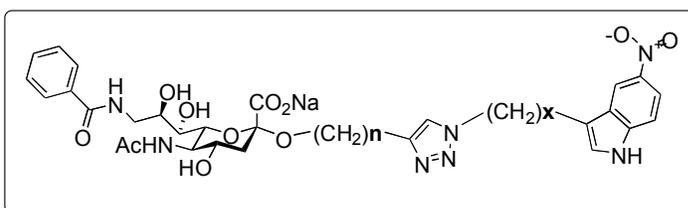
Table 15: K_D values for the precursors **SA1-4** and **NI1-3** precursors.

(SA)	K_D (µM)	(NI)	K_D (µM)
n = 1	76.5	x = 1	
n = 2	63.9	x = 3	
n = 3	47.0		
n = 4	39.6		

The other combinations, **SA1-4NI1**, which were not identified by *in-situ* click chemistry, were found to be inactive against MAG. This illustrates the potential of applying the *in-situ* click chemistry approach to selectively identify a potent hit.

The best hit **SA1NI3** (K_D= 0.19 μM) was found to be 60 times more active than the previously best known compound **12h** (K_D=11.8 μM).

In order to cross check whether the triazole just acts as a linker between two binding sites or contributes to the binding, we synthesized the (*N*-unsubstituted 1,2,3-triazole) sialic acid derivative **42 (Scheme 10)** by a recently reported literature method.[188]

The synthesis of (*N*-unsubstituted 1,2,3-triazole) sialic acid derivative **42** was non-trivial, since the copper (I) catalyzed click chemistry reactions did not work with sodium azide. Other methods of using TMS-azide and/or only sodium azide as the azide component for the click reactions were unsuccessful. Finally applying the reported method[188] from the Sharpless group resulted in a successful synthesis of the *N*-unsubstituted-1,2,3-triazole sialic acid derivative **42**.
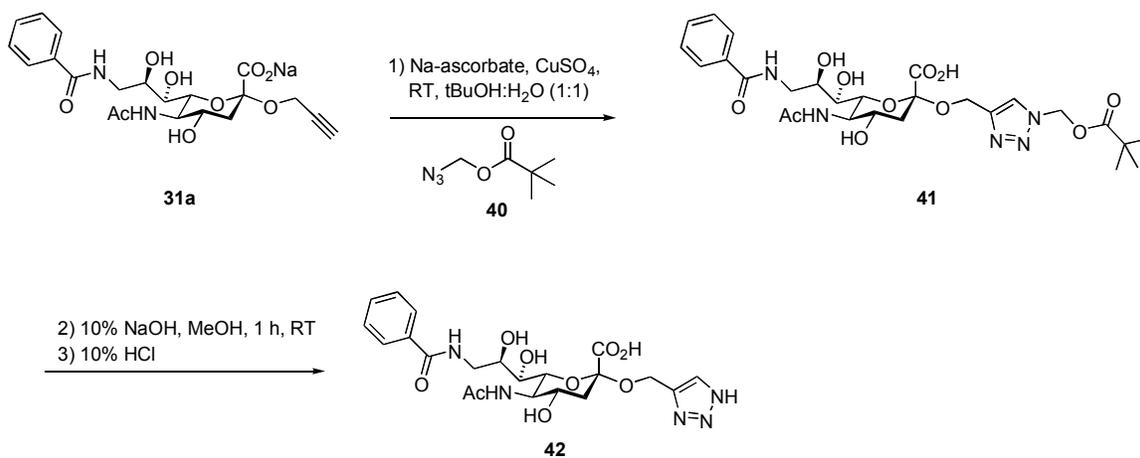
Scheme 10.

Treating chloro-methyl pivaloate with aqueous sodium azide under heterogenous conditions, afforded azido-methyl pivaloate **40** in analytical pure form upon workup. A facile cycloaddition between azido-methyl pivaloate **40** and sialic acid precursor **31a** was achieved using the standard click chemistry parameters and reagents, resulting in 1,4-disubstituted-[1,2,3]-triazole **41** in good yield. The

pivaloate group was easily removed using dilute aqueous sodium hydroxide solution for few minutes to give the 4-Methyl-(5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranoside)-*N*H-1,2,3-triazole **42**.

<div align="center">Table 16: K_D of the unsubstituted triazole 42.</div>
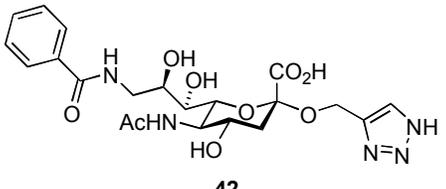
Compound	K_D
  42	11.72 μM

The K_D value of 11.72 μM for **42** indicate that the triazole moiety interacts with the MAG and then thereby contributing to an improved affinity of **SA1NI3** compound.

14. STD-NMR study of SA1NI3:

(were perormed by **Dr. Brian Cutting** from the Institute of Molecular Pharmacy, University of Basel)

After the identification of **SA1NI3** as a ligand with a nanomolar affinity for MAG, a STD-NMR study was performed to elucidate the binding epitope. As seen in **Figure 36**, both aromatic moieties contribute a large STD effect as compared to the sugar moieties. As expected and shown in our previous STD studies with compound **12a**, the benzamide group in both cases is an important epitope binding epitope.

The most interesting information obtained concernes the contribution from the 5-nitro-indole and triazole moieties. The STD values show that the 5-nitro-indole group contributes to a similar extent as the benzamide group. Furthermore it can

be stated that the triazole forms a contact with the MAG probably similar to the reducing end benzyl group as in **12a**. This information on the binding epitopes will be highly valuable for the design of the next generation of mimics.
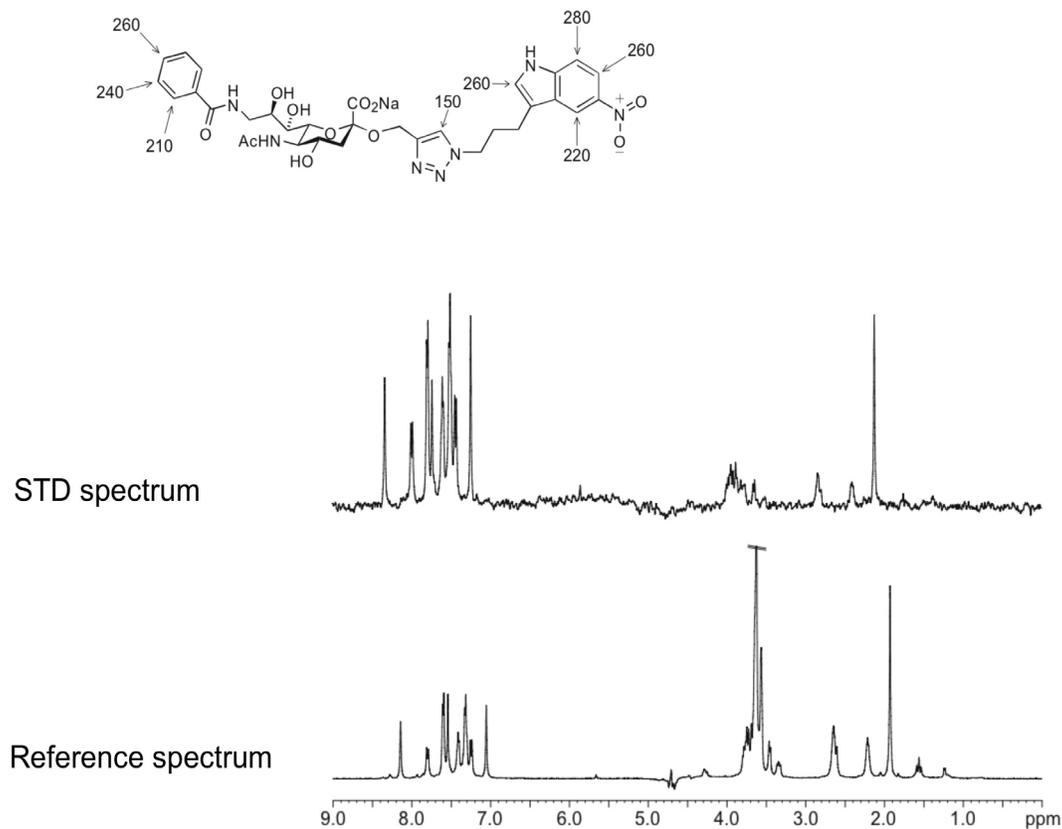
Figure 36: Group epitope mapping and STD-NMR spectra of **SA1NI3.**

CONCLUSION AND OUTLOOK:

15. CONCLUSION

From this study it can be concluded that the substituted mono-sialosides were better ligands for MAG than the natural oligosaccharides. These compounds also have better pharmacokinetic properties due to the presence of aromatic moieties, a low molecular weight, and a reduced number of polar hydroxy functions.

We achieved a strong enhancement in binding potency by a systematic approach. Specifically, starting from trisaccharide **A,** we discovered compound **12a** that exhibits a 700-fold improved affinity (rIP value) for MAG. The necessity of an amide group at the 9-position of sialic acid was proved by the low rIPs for compounds **10** (**Table 7**), **C** (**Table 8**) and the triazole derivatives **13a-f** (**Table 9**). By applying the Topliss operational scheme we could identify the lead compound **12h**, which was found to be 1000-fold (rIP value) more active than the trisaccharide **A**.

For mimicking trisaccharide **A** the disaccharide moiety was replaced by a benzyl group and the 9-position of sialic acid was substituted by a benzamide group.

A (rIP=1)   **12a** (rIP=690)   **12h** (rIP=1074)

10 (rIP=0.05)   **13a-f** (rIP=0.0007-0.29)   **C** (rIP=2.3)
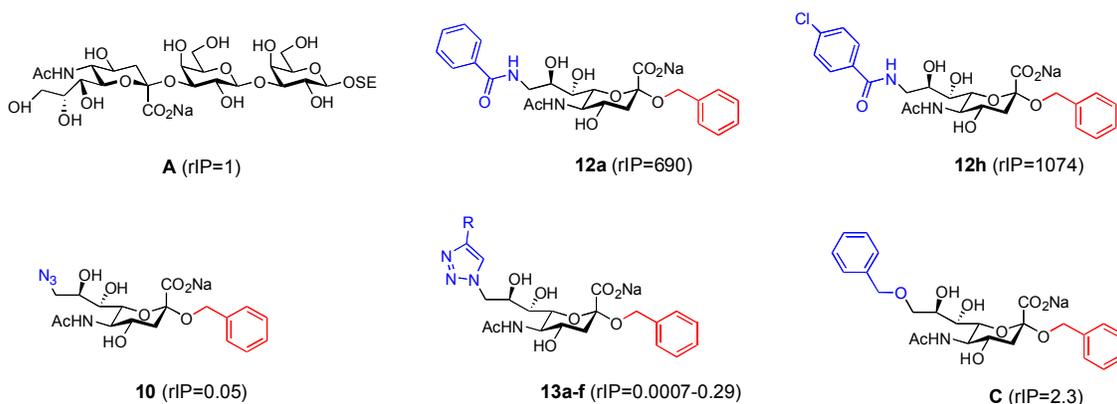
The next generation of ligands was identified by a fragment-based NMR screening and *in-situ* click chemistry. We have termed this approach as a "Receptor-mediated Approach". Using the second binding site screening method by NMR with spin-label **12a***, we could successfully identify a ligand for a second binding site in the vicinity of the first binding site.
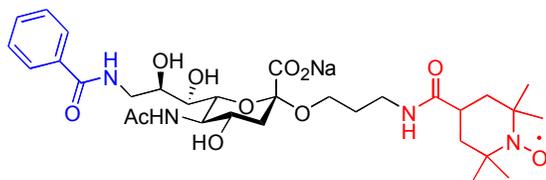
12a*
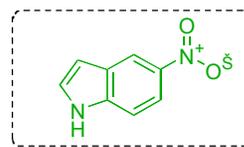
Second binding site ligand

The linking of these two ligands was efficiently achieved using the concept of *in-situ* click chemistry. Finally, the nanomolar compound **SA1NI3** was identified from the *in-situ* click chemistry experiments. The identified compound was scaled up by a copper-catalysed procedure. With the Biacore assay, the compound **SA1NI3** showed an affinity of **190 nM** for MAG.
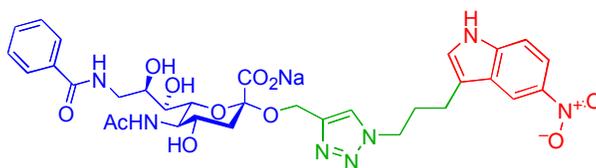
SA1NI3

16. OUTLOOK

Future directions will be based on the current research work. Replacing the benzamide moiety in **SA1NI3** with the *p*-chloro benzamide moiety, as identified by our TOS study, should further improve affinity.
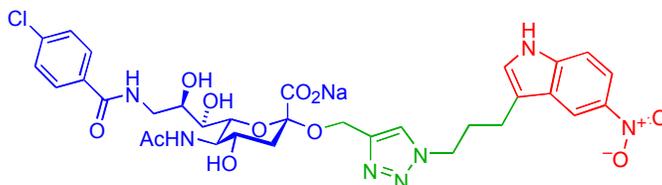
Furthermore, the nitro group in **SA1NI3,** has to be replaced, since it is usually avoided in medicinal chemistry due to its reactivity and instability in a physiological environment, leading e.g. to the formation of a nitroso group. Replacing the nitro functionality with its isosteres will lead to MAG-antagonists with an improved drug-like character.

R1, **R2** = CF₃, Cl, F, COOH...
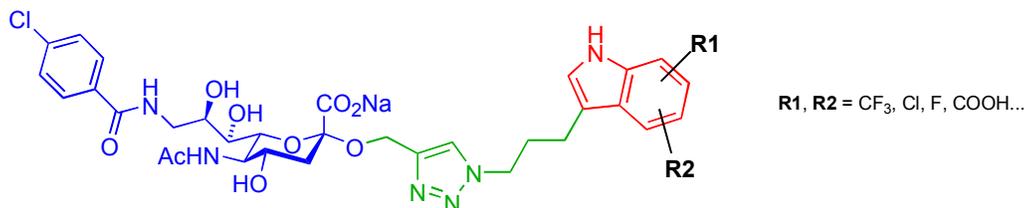
The STD-NMR and Biacore studies indicate that the role of triazole is more than just as a linker. This offers the opportunity to improve bidning with derivatized aromatic moieties.

X = C, N, O, S   **R** = Cl, F, OH, CH₃....
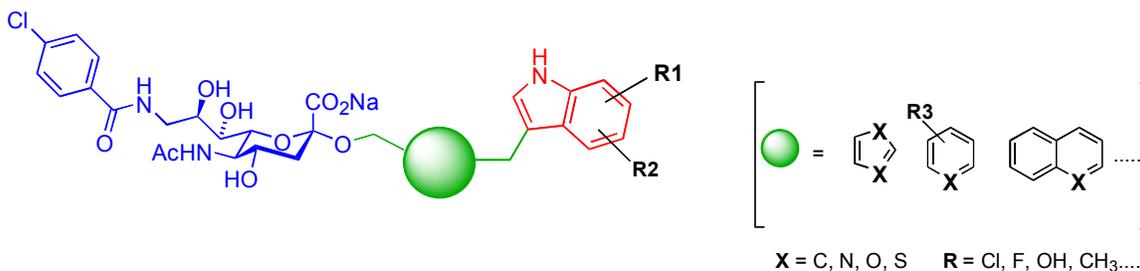
Finally, combining all the optimizations at various, it is possible to design the new generation of MAG anatagonists as shown below. The hydroxamic acid substituion would be interesting from medicinal chemistry point of approach.

R1, **R2** = CF_3, Cl, F, COOH...
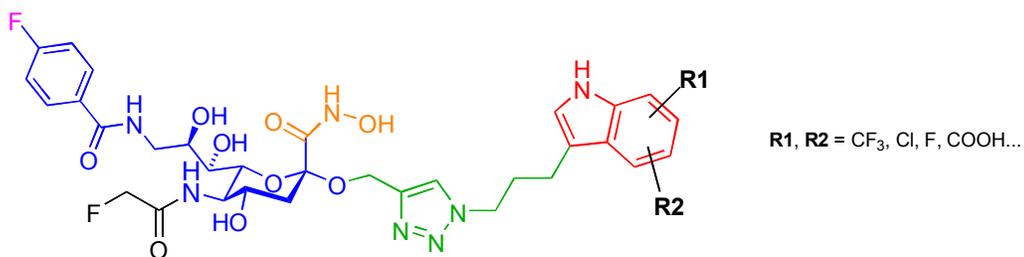
EXPERIMENTAL

Chemistry. NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}CNMR spectra was achieved using 2D methods (COSY, HSQC, TOCSY). Chemical shifts are expressed in ppm using residual CHCl$_3$, CHD$_2$OD and HDO as references. Optical rotations were measured using a Perkin-Elmer Polarimeters 241 and 341. MS analyses were carried out using a Waters Micromass ZQ Detector system. The spectra were recorded in positive ESI mode. 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$_2$SO$_4$). Column chromatography was performed on silica gel (Uetikon, 40-60 mesh). Methanol was dried by refluxing with sodium methoxide and distilled immediately before use. Pyridine was freshly distilled under argon over KOH. Dichloromethane (DCM), dichloroethane (DCE), acetonitrile (ACN), toluene, and benzene were dried by filtration over Al$_2$O$_3$ (Fluka, type 5016 A basic). Molecular sieves (3 Å) were activated in vacuo 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-nonulopyranosid)onate (2).
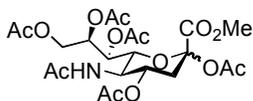
N-Acetyl neuraminic acid **1** (6.18 g, 20.0 mmol) and Amberlyst 15 (5.00 g) were stirred at r.t. in MeOH (300 mL) for 16 h. TLC analysis (DCM/MeOH 7:3) showed completion of the reaction. The reaction mixture was filtered and the residual solid thoroughly washed with MeOH. After evaporation of the solvent, the methyl ester (5.50 g) was obtained and taken as such for the acetylation step. The above product was dissolved in dry pyridine (68 mL) and stirred at 0°C for 15 min under argon before DMAP (323 mg, 2.70 mmol) and Ac$_2$O (76.6 mL, 715 mmol) were added simultaneously at 0°C. After stirring at r.t. for 14 h, TLC analysis

showed completion of the reaction. After evaporation of solvents, chromatography on silica gel (DCM/MeOH 20:1) yielded **2** as a white foam (7.25 g, 81%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.90 (s, 3H, NHAc), 2.04 (m, 10H, H-3a, 3OAc), 2.14 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.66 (dd, *J* = 5.0, 13.5 Hz, 1H, H-3e), 3.79 (s, 3H, CO$_2$CH$_3$), 4.12 (m, 3H, H-5, H-6, H-9a), 4.49 (dd, *J* = 2.5, 12.4 Hz, 1H, H-9b), 5.08 (m, 1H, H-8), 5.26 (m, 1H, H-4), 5.38 (m, 2H, H-7, NH),

Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-thio-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (3).
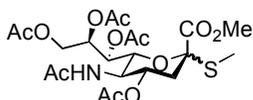
To a stirred solution of **2** (7.20 g, 13.5 mmol) in 1,2-dichloroethane (100 mL) containing activated mol. sieves (3Å) (5.00 g), TMSSMe (2.26 g, 18.9 mmol) and TMSOTf (2.24 g, 10.1 mmol) were added simultaneously. The mixture was stirred under argon at 50°C for 5 h and then for 16 h at r.t. After dilution with DCM (25 mL) the reaction mixture was subsequently washed with sat. aqueous NaHCO$_3$ (100 mL) and water (100 mL), dried over Na$_2$SO$_4$, filtered and concentrated. Purification by column chromatography on silica gel (DCM/MeOH 40:1) yielded **3** (5.75 g, 82%) as a fluffy solid. This was taken as such in the next step.

Methyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α- and β-D-*galacto*-2-nonulopyranosid)onate (4α and 4β).
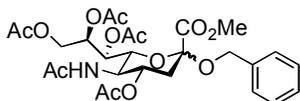
Compound **3** (5.20 g, 10.0 mmol), benzyl alcohol (1.62 g, 15.0 mmol) and 3Å molecular sieves (5 g) were suspended in dry MeCN (100 mL) and stirred under argon with cooling until temperature reached –40°C. NIS (2.68 g, 12.0 mmol) and trifluoromethane sulfonic acid (600 mg, 4.00 mmol) were added successively. After stirring for 30 min, the reaction was warmed to –30°C and stirring was continued at this temperature for additional 14 h. The mixture was diluted with

DCM and filtered through a pad of Celite. After subsequent washing with 20% aqueous $Na_2S_2O_3$ (200 mL) and sat. aqueous $NaHCO_3$ (200 mL), the organic layer was dried over Na_2SO_4, filtered and concentrated to give a syrup. Purification by column chromatography on silica gel (petrol ether/DCM/2-propanol 8:4:1) yielded α-isomer **4a** (2.40 g, 41%) and β-isomer **4b** (1.04 g, 18%).

^{1}H NMR (500 MHz, $CDCl_3$): **4a**: δ 1.90 (s, 3H, NHAc). 2.03 (s, 3H, OAc), 2.03 (m, 4H, H-3a, OAc), 2.05 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.17 (s, 3H, OAc), 2.66 (dd, J = 4.6, 12.9 Hz, 1H, H-3e), 3.67 (s, 3H, CO_2CH_3), 4.09–4.15 (m, 3H, H-5, H-6, H-9a), 4.33 (dd, J = 2.7, 12.4 Hz, 1H, H-9b), 4.43, 4.82 (A, B of AB, J = 12.0 Hz, 2H, CH_2Ph), 4.87 (m, 1H, H-4), 5.21 (d, 1H, NH), 5.35 (dd, J = 2.1, 8.5 Hz, 1H, H-7), 5.47 (m, 1H, H-8), 7.25–7.37 (s, 5H, Ar-H). **4b**: 1.87 (s, 3H, NHAc), 1.96 (m, 4H, H-3a, OAc), 2.01 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.56 (1H, H-3e), 3.73 (s, 3H, CO_2CH_3), 3.99 (dd, J = 2.2, 10.5 Hz), 1H, H-9a), 4.10-4.15 (m, 3H, H-5, H-9b), 4.52 (A, B of AB, J = 11.9 Hz, 2H, CH_2Ph), 4.84 (dd, J = 2.5, 12.5 Hz, 1H, H-6), 5.28-5.41 (m, 4H, H-4, H-7, H-8, NH), 7.36 (m, 5H, Ar-H).

Methyl (benzyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (6):
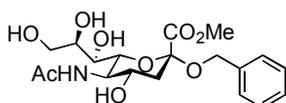
A solution of **4a** (2.38 g, 3.95 mmol) in dry methanol (90 mL) was treated with 1 M methanolic NaOMe (10.5 mL) at r.t. for 2 h. The reaction mixture was neutralized with Amberlyst 15 (H$^+$) ion-exchange resin and filtered through a pad of Celite. The Celite was washed thoroughly with methanol (3 × 5 mL), and the combined filtrates were evaporated to dryness to give **6** as colorless foam (1.40 g, 82%).

^{1}H NMR (500MHz, CD_3OD): δ 1.85 (t, J = 12.6 Hz, 1H, H-3a), 2.05 (s, 3H, NHAc), 2.78 (dd, J = 4.6, 12.8 Hz, 1H, H-3e), 3.58 (dd, J = 1.6, 11.0 Hz, 1H, H-9a), 3.67–3.74 (m, 3H, H-4, H-6, H-9b), 3.81 (s, 3H, CO_2CH_3), 3.83–3.95 (m, 4H,

NH, H-5, H-7, H-8), 4.56, 4.86 (A, B of AB, J = 11.6 Hz, 2H, CH$_2$Ph), 7.28–7.33 (m, 5H, Ar-H).

Methyl [benzyl 5-acetamido-3,5-dideoxy-9-*O*-(4-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid]onate (7):
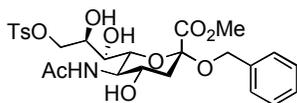
To a solution of **6** (1.20 g, 2.90 mmol) in pyridine was added *p*-TsCl (608 mg, 3.20 mmol) at 0°C. After 2 h, *p*-TsCl (220 mg, 1.16 mmol) was added and stirring continued for 16 h at 5°C. The reaction mixture was warmed to r.t., diluted with methanol (40 mL) and stirring continued for 30 min. After removal of the solvents the remaining syrup was purified by chromatography on silica gel (DCM/MeOH 19:1) to yield **7** as a fluffy solid (929 mg, 56%).

^{1}H NMR (500MHz, CD$_3$OD): δ 1.80 (t, J = 12.7 Hz, 1H, H-3a), 2.04 (s, 3H, NHAc), 2.45 (s, 3H, CH$_3$), 2.74 (dd, J = 4.6, 12.8, Hz, 1H, H-3e), 3.52 (dd, J = 1.5, 8.5 Hz, 1H, H-7), 3.65 (dd, J = 1.5, 10.4 Hz, 1H, H-6), 3.69 (m, 1H, H-4), 3.77 (m, 1H, H-5), 3.79 (s, 3H, CO$_2$CH$_3$), 4.05 (ddd, J = 2.1, 6.4, 9.4 Hz, 1H, H-8), 4.13 (dd, J = 6.4, 10.1 Hz, 1H, H-9a), 4.40 (dd, J = 2.2, 10.1 Hz, 1H, H-9b), 4.49, 4.77 (A, B of AB, J = 11.6 Hz, 2H, CH$_2$Ph), 7.33, 7.45, 7.83 (m, 9H, Ar-H).

Methyl (benzyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid)onate (8):
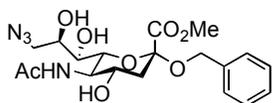
A mixture of **7** (910 mg, 1.60 mmol), crown ether 18-C-6 (169 mg, 0.64 mmol) and NaN$_3$ (520 mg, 8.00 mmol) was stirred in DMF (30 mL) at 60°C for 24 h. TLC analysis (DCM/acetone 1:1) showed completion of the reaction. The mixture was filtered through a pad of Celite and the filtrate was evaporated to dryness. Purification by column chromatography on silica gel (DCM/acetone 7:3) yielded **8** as a white solid (525 mg, 75 %).

^{1}H NMR (500 MHz, CD$_3$OD): δ 1.84 (t, *J* = 12.6 Hz, 1H, H-3a), 2.06 (s, 3H, NHAc), 2.77 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.43 (dd, *J* = 6.2, 12.9 Hz, 1H, H-9a), 3.54 (dd, *J* = 1.4, 8.9 Hz, 1H, H-7), 3.60 (dd, *J* = 2.5, 12.8 Hz, 1H, H-9b), 3.75 (m, 2H, H-4, H-6), 3.81 (s, 3H, CO$_2$CH$_3$), 3.83 (m, 1H, H-5), 4.06 (m, 1H, H-8), 4.57, 4.85 (A, B of AB, *J* = 11.6 Hz, 2H, CH$_2$Ph), 7.36 (m, 5H, Ar-H).

Methyl (benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (9).
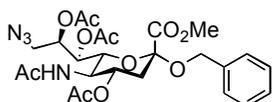
Compound **8** (480 mg, 1.09 mmol) was dissolved in dry pyridine (4.29 g, 54.3 mmol) and stirred at 0°C for 15 min under argon before DMAP (21.4 mg, 0.175 mmol) and Ac$_2$O (4.93 g, 46.0 mmol) were added simultaneously at 0°C. After stirring at r.t. for 14 h, TLC analysis showed completion of the reaction. After evaporation of the solvents, chromatography on silica gel (DCM/MeOH 20:1) yielded **9** as a white foam (470 mg, 73%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.89 (s, 3H, NHAc), 2.03 (s, 3H, OAc), 2.04 (t, *J* = 12.6 Hz, 1H, H-3a), 2.17 (s, 3H, OAc), 2.19 (s, 3H, OAc), 2.67 (dd, *J* = 4.6, 12.9 Hz, 1H, H-3e), 3.28 (dd, *J* = 5.9, 13.5 Hz, 1H, H-9a), 3.59 (dd, *J* = 3.0, 13.5 Hz, 1H, H-9b), 3.70 (s, 3H, CO$_2$CH$_3$), 4.12 (m, 1H, H-5), 4.44, 4.80 (A, B of AB, *J* = 11.9 Hz, 2H, CH$_2$Ph), 4.87 (m, 1H, H-4), 5.19 (m, 1H, NH), 5.36 (m, 3H, H-6, H-7, H-8), 7.31 (m, 5H, Ar-H).

General method I for the synthesis of 9-substituted amides (11a–g): To a solution of **9** (1 eq.) and acid chloride (4 eq.) in toluene, DCM or DCE was added a solution of triphenyl phosphine (2.2 eq.) in DCM, DCE or toluene. After stirring for 1-3 h, the solvent was evaporated. Purification by column chromatography on silica gel (DCM/MeOH 100:1 to 20:1) yielded **12a-12g** as solids.

Methyl (benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11a):
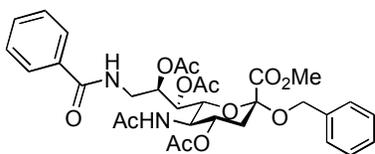
According to the general method **I**, compound **9** (42.0 mg, 0.071 mmol) was reacted with benzoyl chloride (20.0 mg, 0.142 mmol) and triphenyl phosphine (24.0 mg, 0.091 mmol) in toluene (2 mL) for 1 h. After workup **11a** was obtained as a solid (25.0 mg, 54%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.99 (s, 3H, NHAc), 2.05 (m, 4H, H-3a, OAc), 2.15 (s, 3H, OAc), 2.27 (s, 3H, OAc), 2.67 (dd, J = 4.6, 12.8 Hz, 1H, H-3e), 2.96 (dd, J = 3.3, 11.5 Hz, 1H, H-9a), 3.65 (s, 3H, OCH$_3$), 4.06 (d, J = 10.7 Hz, 1H, H-6), 4.21 (q, J = 10.7 Hz, 1H, H-5), 4.39 (m, 1H, H-9b), 4.44, 4.83 (A, B of AB, J = 12.0 Hz, 2H, CH$_2$Ph), 4.85 (m, 1H, H-4), 5.17 (m, 2H, NH-5, H-7), 5.33 (m, 1H, H-8), 7.09 (m, 1H, NH-9), 7.33–7.44, 7.84 (m, 10H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-(4-biphenylcarboxamido)-3,5,9-trideoxy-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (11b).
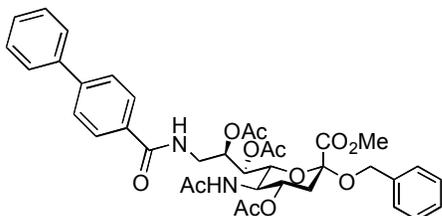
According to the general method **I**, compound **9** (50.0 mg, 0.084 mmol) was reacted with biphenyl-4-carbonyl chloride (26.2 mg, 0.10 mmol) and triphenyl phosphine (36.7 mg, 0.17 mmol) in toluene (2 mL) for 2 h. After workup **11b** was obtained as a solid (14.0 mg, 24%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.91 (s, 3H, NHAc), 2.06 (m, 3H, H-3a, OAc), 2.16 (s, 3H, OAc), 2.28 (s, 3H, OAc), 2.67 (dd, J = 4.6, 12.7 Hz, 1H, H-3e), 2.98 (m, J = 1H, H-9a), 3.65 (s, 3H, OCH$_3$), 4.07 (dd, J = 2.0, 10.8 Hz, 1H, H-6), 4.22 (dd, J = 10.3, 10.8 Hz, 1H, H-5), 4.41 (m, 1H, H-9b), 4.45, 4.85 (A, B of AB, J = 12.0 Hz, 2H, CH$_2$Ph), 4.86 (m, 1H, H-4), 5.20 (m, 2H, H-7, NH-5), 5.35 (m, 1H, H-8), 7.15, (m, 1H, NH-9), 7.33, 7.39, 7.47, 7.62, 7.67, 7.91 (m, 14H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-(2-biphenylcarboxamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (11c).
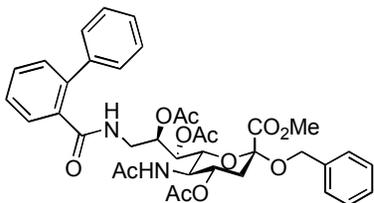
According to the general method **I**, compound **9** (50.0 mg, 0.084 mmol) was reacted with biphenyl-2-carbonyl chloride (72.5 mg, 0.336 mmol) and triphenyl phosphine (48.0 mg, 0.183 mmol) in DCM (2 mL) for 1 h. After workup **11c** was obtained as a solid (40.0 mg, 66%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 2.02 (m, 1H, H-3a, NHAc), 2.08 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.17 (s, 3H, OAc), 2.63–2.70 (m, 2H, H-3e, H-9a), 3,69 (s, 3H, OCH$_3$), 3.94 (dd, *J* = 2.3, 10.7 Hz, 1H, H-6), 4.06-4.15 (m, 2H, H-5, H-9b), 4.39, 4.79 (A, B of AB, *J* = 12.0 Hz, 2H, CH$_2$Ph), 4.67 (dd, *J* = 2.2, 9.4 Hz, 1H, H-7), 4.83 (m, 1H, H-4), 4.99 (d, 1H, NH-5), 5.12 (m, 1H, H-8), 5.99 (m, 1H, NH-9), 7.31–7.59 (m, 1H, Ar-H).

Methyl (benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5,9-trideoxy-9-orotinoylamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11d).
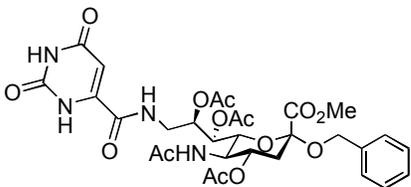
According to the general method **I**, compound **9** (50.0 mg, 0.084 mmol) was reacted with orotinoyl chloride (63.8 mg, 0.336 mmol) and triphenyl phosphine (48.0 mg, 0.183 mmol) in DCM (2 mL) for 1 h. After workup **11d** was obtained as a solid (40.0 mg, 70%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.92 (s, 3H, NHAc), 2.05 (m, 4H, H-3a, OAc), 2.13 (s, 3H, OAc), 2.26 (s, 3H, OAc), 2.67 (dd, *J* = 4.6, 12.7 Hz, 1H, H-3e), 2.93 (m, 1H, H-9a), 3.99 (s, 3H, OCH$_3$), 4.03 (dd, *J* = 2.0, 10.7 Hz, 1H, H-6), 4.21 (m, 1H, H-5), 4.27 (m, 1H, H-9b), 4.41, 4.79 (A, B of AB, *J* = 12.0 Hz, 2H, CH$_2$Ph), 4.84 (m, 1H, H-4), 5.04 (dd, *J* = 2.0, 9.9 Hz, 1H, H-7), 5.32 (m, 2H, NH-5, H-8), 6.08

(s, C<u>H</u>=C), 6.40 (s, C=C<u>H</u>) 7.32 (s, 5H, Ar-H), 7.49 (m, 1H, NH-9), 8.65 (m, 1H, allylic NH), 8.82 (s, 1H, NH).

Methyl (benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-butyrylamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11e)
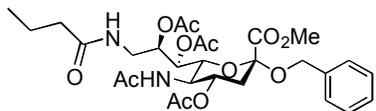
According to the general method **I**, compound **9** (50.0 mg, 0.084 mmol) was reacted with butyryl chloride (35.8 mg, 0.336 mmol) and triphenyl phosphine (48.0 mg, 0.183 mmol) in DCM (2 mL) for 3 h. After workup **11e** was obtained as a solid (43 mg, 84%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 0.96 (t, *J* = 8.3 Hz, 3H, H-4′), 1.63–2.68 (m, 2H, H-3′), 1.91 (s, 3H, NHAc), 2.04 (m, 4H, H-3a, OAc), 2.13 (s, 3H, OAc), 2.17 (t, *J* = 7.4 Hz, 2H, H-2′), 2.21 (s, 3H, OAc), 2.66 (dd, *J* = 4.5, 12.8 Hz, 1H, H-3e), 2.78 (m, 1H, H-9a), 3.66 (s, 3H, CO$_2$CH$_3$), 4.04–4.20 (m, 3H, H-5, H-6, H-9), 4.42, 4.82 (A, B of AB, *J* = 12.0 Hz, 2H, CH$_2$Ph), 4.86 (m, 1H, H-4), 5.09 (d, *J* = 10.6 Hz, 1H, H-7), 5.23 (m, 2H, H-8, NH-5), 6.17 (m, 1H, NH-9), 7.32 (m, 5H, Ar-H),

Methyl (benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5,9-trideoxy-9-hexanoylamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12f).
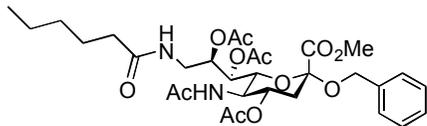
According to the general method **I**, compound **9** (50.0 mg, 0.084 mmol) was reacted with caproyl chloride (45.6 mg, 0.336 mmol) and triphenyl phosphine (48.0 mg, 0.183 mmol) in DCM (2 mL) for 3 h. After workup **11f** was obtained as a solid (26 mg, 49%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 0.90 (t, *J* = 6.9 Hz, 3H, H-6′), 1.30 (m, 4H, H-4′, H-5′), 1.62 (m, 2H, H-3′), 1.91 (s, 3H, NHAc), 2.04 (m, 4H, H-3a, OAc), 2.13 (s, 3H, OAc), 2.18 (t, *J* = 6.9 Hz, 2H, H-2′), 2.21 (s, 3H, OAc), 2.56 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.79 (m, 1H, H-9a), 3.66 (s, 3H, CO$_2$CH$_3$), 4.09 (m, 2H, H-6, H-9b), 4.18 (q, *J* = 10.4 Hz, 1H, H-5), 4.42, 4.82 (A, B of AB, *J* = 12.0 Hz, 2H, CH$_2$Ph),

4.86 (ddd, *J* = 5.7, 6.5, 10.4, Hz, 1H, H-4), 5.01 (dd, *J* = 2.1, 9.7 Hz, 1H, H-7), 5.24 (m, 2H, H-8, NH-5), 6.14 (m, 1H, NH-9), 7.26–7.33 (m, 5H, Ar-H).

Methyl (benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-decanoylamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11g).
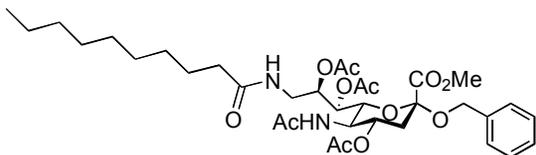
According to the general method **I**, compound **9** (50.0 mg, 0.084 mmol) was reacted with decanoyl chloride (65.2 mg, 0.336 mmol) and triphenyl phosphine (48.0 mg, 0.183 mmol) in DCM (2 mL) for 3 h. After workup **11g** was obtained as a solid (30 mg, 51%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 0.87 (t, *J* = 6.8 Hz, 3H, H-10′), 1.26–1.31 (m, 14H, H-4′, H-5′, H-6′, H-7′, H-8′, H-9′), 1.60 (m, 2H, H-3′), 1.91 (s, 3H, NHAc), 2.04 (m, 4H, H-3a, OAc), 2.13 (s, 3H, OAc), 2.18 (t, *J* = 7.9 Hz, 2H, H-2′), 2.21 (s, 3H, OAc), 2.66 (dd, *J* = 4.5, 12.7 Hz, 1H, H-3e), 2.79 (m, 1H, H-9a), 3.65 (s, 3H, CO$_2$CH$_3$), 4.06–4.20 (m, 3H, H-5, H-6, H-9b,), 4.42, 4.82 (A, B of AB, *J* = 12.0 Hz, 2H, CH$_2$Ph), 4.86 (ddd, *J* = 4.9, 6.2, 10.3 Hz, 1H, H-4), 5.09 (d, *J* = 8.8 Hz, 1H, H-7), 5.18–5.25 (m, 2H, H-8, NH-5), 6.13 (m, 1H, NH-9), 7.27–7.33 (m, 5H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (11h).
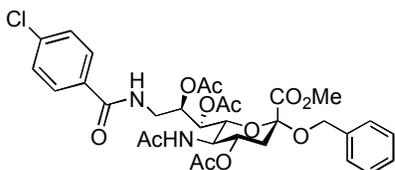
According to the general method **I**, compound **9** (40.0 mg, 0.070 mmol) was reacted with *p*-chloro benzoyl chloride (49.0 mg, 0.280 mmol) and triphenyl phosphine (40.3 mg, 0.154 mmol) in DCE (2 mL) for 14 h. After workup **11h** was obtained as a solid (32.0 mg, 68%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.89 (s, 3H, NHAc), 2.04 (m, 4H, H-3a, OAc), 2.14 (s, 3H, OAc), 2.26 (s, 3H, OAc), 2.67 (dd, J = 4.6, 12.8 Hz, 1H, H-3e), 2.96 (m, 1H, H-9a), 3.64 (s, 3H, OCH$_3$), 4.06 (dd, J = 2.0, 10.7 Hz, 1H, H-6), 4.20 (q, J = 10.4, 1H, H-5), 4.35 (m, 1H, H-9b), 4.43 (A of AB, J = 12.0 Hz, 1H, CH$_2$Ph), 4.83 (m, 2H, H-4, B of AB CH$_2$Ph), 5.15 (dd, J = 2.0, 9.9 Hz, 1H, H-7), 5.27 (d, J = 10.1 Hz, 1H, NH-5), 5.34 (m, 1H, H-8), 7.09 (m, 1H, NH-9), 7.25-7.43, 7.77 (m, 9H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5,9-trideoxy-9-(4-methoxybenzamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (11i).
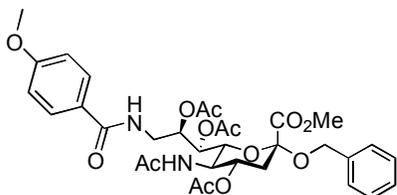
According to the general method **I**, compound **9** (40.0 mg, 0.070 mmol) was reacted with *p*-anisoyl chloride (47.6 mg, 0.280 mmol) and triphenyl phosphine (40.3 mg, 0.154 mmol) in DCE (2 mL) for 14 h. After workup **11i** was obtained as a solid (25.0 mg, 53%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.89 (s, 3H, NHAc), 2.04 (m, 4H, H-3a, OAc), 2.14 (s, 3H, OAc), 2.25 (s, 3H, OAc), 2.67 (dd, J = 4.6, 12.7 Hz, 1H, H-3e), 2.95 (m, 1H, H-9a), 3.64 (s, 3H, CO$_2$CH̲$_3$), 3.84 (s, 3H, PhOCH̲$_3$), 4.06 (dd, J = 2.1, 10.7 Hz, 1H, H-6), 4.21 (q, J = 10.4, 1H, H-5), 4.35 (m, 1H, H-9b), 4.44 (A of AB, J = 11.9 Hz, 1H, CH$_2$Ph), 4.84 (m, 2H, H-4, B of AB CH$_2$Ph), 5.18 (dd, J = 2.0, 9.8 Hz, 1H, H-7), 5.26 (d, J = 10.1 Hz, 1H, NH-5), 5.33 (m, 1H, H-8), 6.92 (m, 2H, Ar-H), 6.96 (m, 1H, NH-9), 7.247.34, 7.79 (m, 7H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5,9-trideoxy-9-(4-methylbenzamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (11j).
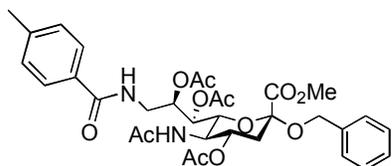
According to the general method **I**, comound **9** (40.0 mg, 0.070 mmol) was reacted with *p*-toluyl chloride (43.1 mg, 0.280 mmol) and triphenyl phosphine (40.3 mg, 0.154 mmol) in DCE (2 mL) for 14 h. After workup **11j** was obtained as a solid (26.0 mg, 56%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.89 (s, 3H, NHAc), 2.04 (m, 4H, H-3a, OAc), 2.14 (s, 3H, OAc), 2.26 (s, 3H, OAc), 2.39 (s, 3H, Ph-C$\underline{H}_3$), 2.67 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.96 (m, 1H, H-9a), 3.64 (s, 3H, CO$_2$CH$_3$), 4.06 (dd, *J* = 1.9, 10.8 Hz, 1H, H-6), 4.20 (q, *J* = 10.4, 1H, H-5), 4.36 (m, 1H, H-9b), 4.43 (A of AB, *J* = 12.0 Hz, 1H, CH$_2$Ph), 4.84 (m, 2H, H-4, B of AB CH$_2$Ph), 5.17 (dd, *J* = 1.9, 9.8 Hz, 1H, H-7), 5.24 (d, *J* = 10.1 Hz, 1H, NH-5), 5.33 (m, 1H, H-8), 7.04 (m, 1H, NH-9), 7.22-7.33, 7.73 (m, 9H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-(3,4-dichlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (11k).
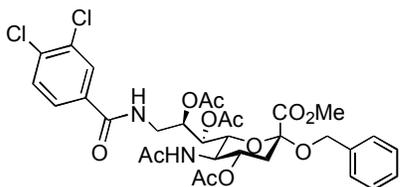
According to the general method **I**, compound **9** (40.0 mg, 0.070 mmol) was reacted with *m,p*-dichlorobenzoyl chloride (58.5 mg, 0.280 mmol) and triphenyl phosphine (40.3 mg, 0.154 mmol) in DCE (2 mL) for 14 h. After workup **11k** was obtained as a solid (31.0 mg, 63%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.91 (s, 3H, NHAc), 2.04 (m, 4H, H-3a, OAc), 2.14 (s, 3H, OAc), 2.27 (s, 3H, OAc), 2.67 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.95 (m, 1H, H-9a), 3.65 (s, 3H, OCH$_3$), 4.06 (d, *J* = 2.0, 10.7 Hz, 1H, H-6), 4.22 (q, *J* = 10.4, 1H, H-5), 4.35 (m, 1H, H-9b), 4.43 (A of AB, *J* = 12.0 Hz, 1H, CH$_2$Ph), 4.85 (m, 2H, H-4, B of AB, CH$_2$Ph), 5.14 (dd, *J* = 2.1, 9.9 Hz, 1H, H-7), 5.25 (d, *J* = 10.1 Hz, 1H, NH-5), 5.33 (m, 1H, H-8), 7.12 (m, 1H, NH-9), 7.33, 7.51, 7.62, 7.94 (m, 8H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-(3-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (11l).
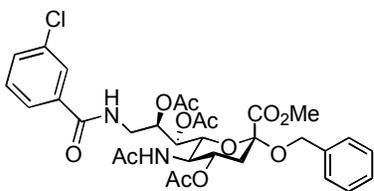
According to the general method **I**, compound **9** (40.0 mg, 0.070 mmol) was reacted with *m*-chlorobenzoyl chloride (49.0 mg, 0.280 mmol) and triphenyl phosphine (40.3 mg, 0.154 mmol) in DCE (2 mL) for 14 h. After workup **11l** was obtained as a solid (28.0 mg, 59%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.84 (s, 3H, NHAc), 1.99 (m, 4H, H-3a, OAc), 2.07 (s, 3H, OAc), 2.19 (s, 3H, OAc), 2.60 (dd, *J* = 4.6, 12.9 Hz, 1H, H-3e), 2.87 (m, 1H, H-9a), 3.57 (s, 3H, CO$_2$CH$_3$), 3.99 (d, *J* = 10.7 Hz, 1H, H-6), 4.14 (q, *J* = 10.7 Hz 1H, H-5), 4.29 (m, 1H, H-9b), 4.36 (A of AB, *J* = 12.0 Hz, 1H, CH$_2$Ph), 4.77 (m, 2H, H-4, B of AB, CH$_2$Ph), 5.09 (d, *J* = 9.9 Hz 1H, H-7), 5.22 (m, 2H, H-8, NH-5), 7.04 (m, 1H, NH-9), 7.14-7.41, 7.60, 7.76 (m, 9H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5,9-trideoxy-9-(4-trifluoromethylbenzamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (11m).
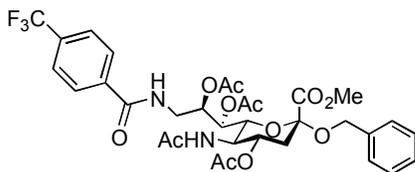
According to the general method **I**, compound **9** (40.0 mg, 0.070 mmol) was reacted with *p*-trifluoromethyl-benzoyl chloride (58.2 mg, 0.280 mmol) and triphenyl phosphine (40.3 mg, 0.154 mmol) in DCE (2 mL) for 14 h. After workup **11m** was obtained as a solid (30.0 mg, 60%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.82 (s, 3H, NHAc), 1.97 (m, 4H, H-3a, OAc), 2.07 (s, 3H, OAc), 2.20 (s, 3H, OAc), 2.60 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.92 (m, 1H, H-9a), 3.57 (s, 3H, CO$_2$CH$_3$), 3.99 (m, 1H, H-6), 4.14 (q, *J* = 10.4, 1H, H-5), 4.29 (m, 1H, H-9b), 4.36 (A of AB, *J* = 12.0 Hz, 1H, CH$_2$Ph), 4.76 (m, 2H, H-4, B of AB, CH$_2$Ph), 5.08 (dd, *J* = 2.1, 9.9 Hz, 1H, H-7), 5.29 (m, 2H, H-8, NH-5), 7.13-7.26, 7.63, 7.86 (m, 10H, NH-9, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-(2,4-dichlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (11n).
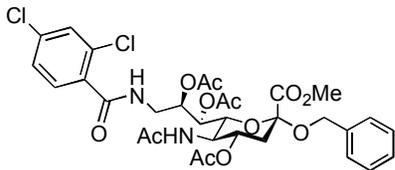
According to the general method **I**, compound **9** (40.0 mg, 0.070 mmol) was reacted with *o,p*-dichlorobenzoyl chloride (58.5 mg, 0.280 mmol) and triphenyl phosphine (40.3 mg, 0.154 mmol) in DCE (2 mL) for 14 h. After workup **11n** was obtained as a solid (31.0 mg, 62%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.82 (s, 3H, NHAc), 1.97 (m, 4H, H-3a, OAc), 2.09 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.60 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.87 (m, 1H, H-9a), 3.59 (s, 3H, CO$_2$CH$_3$), 4.01 (dd, *J* = 2.0, 10.8 Hz, 1H, H-6), 4.11 (q, *J* = 10.4 Hz, 1H, H-5), 4.29 (m, 1H, H-9b), 4.37, 4.76 (A, B of AB, *J* = 12.0 Hz, 2H, CH$_2$Ph), 4.80 (m, 1H, H-4), 5.11 (dd, *J* = 1.9, 9.7 Hz 1H, H-7), 5.23 (m, 2H, H-8, NH-5), 6.84 (m, 1H, NH-9), 7.18-7.26, 7.35, 7.49 (m, 8H, Ar-H).

Methyl [benzyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5,9-trideoxy-9-(4-nitrobenzamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (11o).
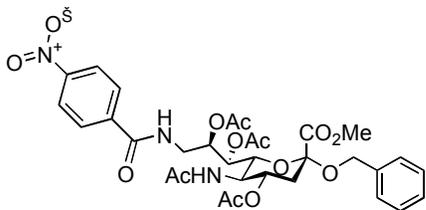
According to the general method **I**, compound **9** (40.0 mg, 0.070 mmol) was reacted with *p*-nitrobenzoyl chloride (51.8 mg, 0.280 mmol) and triphenyl phosphine (40.3 mg, 0.154 mmol) in DCE (2 mL) for 14 h. After workup **11o** was obtained as a solid (28.0 mg, 58%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.82 (s, 3H, NHAc), 1.99 (m, 4H, H-3a, OAc), 2.08 (s, 3H, OAc), 2.20 (s, 3H, OAc), 2.60 (dd, *J* = 4.6, 12.7 Hz, 1H, H-3e), 2.93 (m, 1H, H-9a), 3.58 (s, 3H, CO$_2$CH$_3$), 3.99 (dd, *J* = 2.0, 10.7 Hz, 1H, H-6), 4.15 (q, *J* = 10.4 Hz, 1H, H-5), 4.30 (m, 1H, H-9b), 4.36 (A of AB, *J* = 11.9 Hz, 1H, CH$_2$Ph),

4.76 (m, 2H, H-4, B of AB, CH_2Ph), 5.08 (dd, J = 2.0, 9.8 Hz 1H, H-7), 5.27 (m, 2H, H-8, NH-5), 7.18-7.30, 7.91, 8.20 (m, 10H, NH-9, Ar-H).

General method II for the saponification of final compounds (5, 6, 11, 12a-g).

To a solution of protected compounds **4a/b**, **9**, **11a-g** (25-50 mg) in methanol (2 mL) was added 10% aq. NaOH (0.2 mL). The mixture was stirred at r.t. for 3 h. The solution was concentrated and the residue was purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 ion-exchange chromatography (Na^+ type), and P2 size exclusion chromatography to afford **5**, **6, 11, and 12a-12g** as colorless solids after a final lyophilization from water.

Sodium (benzyl 5-acetamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (5).
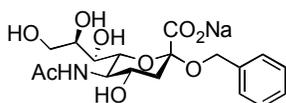
According to the general method II, compound **4a** (50.0 mg, 0.086 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After workup and purification **5** was obtained as a fluffy solid (47 mg, 94%).

$[\alpha]_D^{25}$ = - 4.3 (*c* = 1.00, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.60 (t, J = 11.9 Hz, 1H, H-3a), 1.89 (s, 3H, NHAc), 2.68 (dd, J = 3.5, 12 Hz, 1H, H-3e), 3.49-3.77 (m, 7H, H-4, H-5, H-6, H-7, H-8, H-9), 4.42, 4.65 (A, B of AB, J = 11.0 Hz, 2H, CH_2Ph), 7.30 (m, 5H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 22.4 (NHCO$\underline{C}H_3$), 40.9 (C-3), 52.2 (C-5), 62.9 (C-9), 67.5 ($\underline{C}H_2Ph$), 68.5 (C-7), 68.7 (C-4), 72.0 (C-8), 73.1 (C-6), 101.3 (C-2), 128.7, 129.0, 129.1, 137.4 (6C, C_6H_5), 173.9, 175.4 (2XCO).

Sodium (benzyl 5-acetamido-3,5,9-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (6).
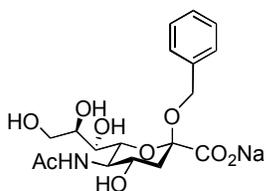
According to the general method II, compound **4b** (50.0 mg, 0.086 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After workup and purification **6** was obtained as a fluffy solid (34 mg, 94%).

$[\alpha]_D^{25}$ = - 1.3 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.68 (t, *J* = 12.1 Hz, 1H, H-3a), 1.90 (s, 3H, NHAc), 2.38 (dd, *J* = 4.7, 13.1 Hz, 1H, H-3e), 3.57 (d, *J* = 9.6 Hz, 1H, H-7), 3.67 (dd, *J* = 6.5, 12.0 Hz, 1H, H-9a), 3.83-3.97 (m, 4H, H-5, H-6, H-8, H-9b), 4.02 (m, 1H, H-4), 4.27, 4.60 (A, B of AB, *J* = 10.1 Hz, 2H, CH_2Ph), 7.39 7.47 (m, 5H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 23.6 (NHCO$\underline{C}$H$_3$), 40.3 (C-3), 52.4 (C-5), 63.9 (C-9), 65.3 ($\underline{C}$H$_2$Ph), 67.4 (C-4), 68.6 (C-7), 70.3 (C-8), 70.6 (C-6), 100.5 (C-2), 128.7, 129.1, 129.2, 137.5 (6C, C_6H_5), 175.1, 181.9 (2XCO).

Sodium (benzyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (10).
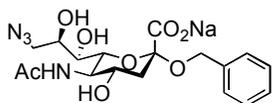
According to the general method **II**, compound **8** (25.0 mg, 0.045 mmol) was deprotected by treatment with 10% NaOH (0.1 mL). After the workup and purification **10** was obtained as a fluffy solid (17 mg, 85%).

$[\alpha]_D^{25}$ = - 3.6 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.67 (t, *J* = 12.2 Hz, 1H, H-3a), 2.03 (s, 3H, NHAc), 2.76 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 3.46 (dd, *J* = 6.1, 13.2 Hz, 1H, H-9a), 3.57 (dd, *J* = 1.8, 9.1 Hz, 1H, H-7), 3.63 (dd, *J* = 2.6, 13.2 Hz, 1H, H-9b), 3.68 (ddd, *J* = 4.1, 4.6, 11.8 Hz, 1H, H-4), 3.74 (d, *J* = 10.4 Hz, 1H, H-6), 3.79-3.83 (m, 2H, H-5, H-8), 4.53, 4.72 (A, B of AB, *J* = 11.0 Hz, 2H, CH_2Ph), 7.41 (s, 5H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 20.5 (NHCO$\underline{C}$H$_3$), 39.1 (C-3), 50.4 (C-5), 51.5 (C-9), 65.8 ($\underline{C}$H$_2$Ph), 66.8 (C-4), 67.4 (C-7), 68.8 (C-8), 71.0 (C-6), 99.6 (C-2), 126.8, 127.2, 127.2, 135.7 (6C, C_6H_5), 172.0, 173.6 (2XCO).

Sodium (benzyl 5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12a).
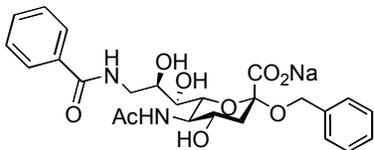
According to the general method II, compound **11a** (25.0 mg, 0.038 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12a** was obtained as a fluffy solid (19 mg, 95 %).

$[\alpha]_D^{25}$ = - 2.0 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.65 (t, *J* = 11.8 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.77 (dd, *J* = 3.5, 11.8 Hz, 1H, H-3e), 3.46-3.52 (m, 2H, H-7, H-9a), 3.68 (m, 1H, H-4), 3.75-3.84 (m, 4H, H-5, H-6, H-8, H-9b), 4.53, 4.70 (A, B of AB, *J* = 11.2 Hz, 2H, CH_2Ph), 7.27-7.39, 7.52, 7.60, 7.78 (m, 10H, Ar-H); ^{13}CNMR (125 MHz, D_2O): δ 21.5 (NHCO$\underline{C}$H3), 40.1 (C-3), 42.1 (C-9), 51.4 (C-5), 66.9 ($\underline{C}$H$_2$Ph), 67.8 (C-4), 69.4 (C-7), 69.8 (C-8), 72.2 (C-6), 101.0 (C-2), 126.6, 127.7, 128.2, 128.3, 131.7, 133.2, 136.8 (12C, 2C_6H_5), 170.7, 172.5, 174.6 (3XCO).

Sodium [benzyl 5-acetamido-9-(4-biphenylcarboxamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12b).
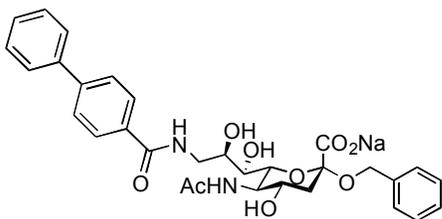
According to the general method II, compound **11b** (14.0 mg, 0.019 mmol) was deprotected by treatment with 10% NaOH (0.1 mL). After the workup and purification **12b** was obtained as a fluffy solid (7.0 mg, 64%).

$[\alpha]_D^{25}$ = - 4.8 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.69 (t, *J* = 12.2 Hz, 1H, H-3a), 2.01 (s, 3H, NHAc), 2.77 (dd, *J* = 4.6, 12.2, Hz, 1H, H-3e), 3.44 (dd, *J* = 8.5, 14.7, Hz, 1H, H-9a), 3.54 (d, *J* = 8.5 Hz, 1H, H-7), 3.68 (m, 1H, H-4), 3.81 (m, 4H, H-5, H-6, H-8, H-9b), 4.48, 4.68 (A, B of AB, *J* = 11.2 Hz, 2H, CH_2Ph),

7.22-7.32, 7.39, 7.54, 7.72 (m, 14H, Ar-H); ^{13}C NMR (125MHz, D$_2$O): δ 22.3 (NHCO$\underline{C}$H$_3$), 40.5 (C-3), 42.5 (C-9), 52.2 (C-5), 67.5 ($\underline{C}$H$_2$Ph), 68.6 (C-4), 70.4 (C-7), 70.8 (C-8), 73.0 (C 6), 101.5 (C-2),127.3, 127.9, 128.4, 128.6, 128.8, 128.9, 129.4, 132.5, 137.4, 139.6, 144.2 (18C, 2XC$_6$H$_5$, C$_6$H$_4$), 171.5, 174.6, 176.3 (3CO).

Sodium [benzyl 5-acetamido-9-(2-biphenylcarboxamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12c).
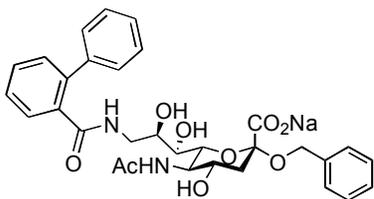
According to the general method II, compound **11c** (40 mg, 0.055 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification, **12c** was obtained as a fluffy solid (32 mg, 97%).

$[\alpha]_D^{25}$ = - 2.7 (*c* = 1.00, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.51 (t, *J* = 11.4 Hz, 1H, H-3a), 1.92 (s, 3H, NHAc), 2.63 (dd, *J* = 4.3, 10.5 Hz, 1H, H-3e), 2.97 (dd, *J* = 9.0, 13.9 Hz, 1H, H-9a), 3.27 (d, *J* = 9.1 Hz, 1H, H-7), 3.47 (m, 1H, H-8), 3.57 (m, 4H, H-4, H-5, H-6, H-9b), 4.36, 4.58 (A, B of AB, *J* = 10.7 Hz, 2H, CH$_2$Ph), 6.93, 7.02 7.07, 7.18-7.34 (m, 14H, Ar-H); ^{13}C NMR (125 MHz, D$_2$O): δ 22.4 (NHCO$\underline{C}$H$_3$), 40.7 (C-3), 43.5 (C-9), 52.2 (C-5), 67.5 ($\underline{C}$H$_2$Ph), 68.6 (C-4), 70.2 (C-8), 70.4 (C-7), 72.8 (C-6), 101.2 (C-2), 127.9, 128.0, 128.1, 128.7, 128.9, 129.0, 129.3, 130.5, 130.9, 135.1, 137.2, 129.9, 140.1 (18C, 2C$_6$H$_5$, C$_6$H$_4$), 173.5, 173.8, 175.4 (3CO).

Sodium (benzyl 5-acetamido-3,5,9-trideoxy-9-orotinoylamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12d).
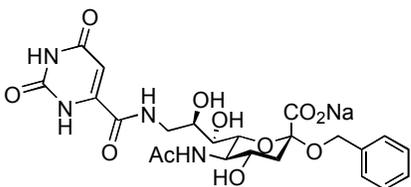
According to the general method II, compound **11d** (40 mg, 0.059 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12d** was obtained as a fluffy solid (32.0 mg, 96%).

$[\alpha]_D^{25}$ = - 3.7 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.69 (t, *J* = 12.1 Hz, 1H, H-3a), 2.01 (s, 3H, NHAc), 2.76 (dd, *J* = 4.5, 12.4 Hz, 1H, H-3e), 2.97 (m, 1H, H-9a), 3.44–3.52 (m, 2H, H-7, H-9b), 3.66–3.84 (m, 4H, H-4, H-5, H-6, H-8), 4.54, 4.69 (A, B of AB, *J* = 11.3 Hz, 2H, CH_2Ph), 6.07, 6.22(2s, 2H, orotinoyl-H), 7.31-7.41 (m, 5H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 23.6 (NHCO$\underline{C}$H3), 42.1 (C 3), 44.9 (C-9), 52.2 (C-5), 67.7 ($\underline{C}$H$_2$Ph), 68.6 (C-4), 70.1 (C-7), 70.4 (C-8), 72.9 (C-6), 98.2 (HN-C=$\underline{C}$-), 100.8 (HN-$\underline{C}$=C-), 128.5, 128.9, 128.9, 129.0, 129.1, 137.7 (6C, C_6H_5), 161.4, 166.7, 174.0, 176.0, 182.0 (5CO).

Sodium (benzyl 5-acetamido-9-butyrylamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12e).
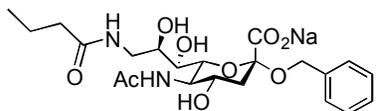
According to the general method II, compound **11e** (42 mg, 0.069 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12e** was obtained as a fluffy solid (32 mg, 96%).

$[\alpha]_D^{25}$ = - 1.2 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.88 (t, *J* = 7.4 Hz, 3H, H 4′), 1.59 (m, 2H, H-3′), 1.68 (t, *J* = 12.2 Hz, 1H, H-3a), 2.01 (s, 3H, NHAc), 2.24 (t, *J* = 7.3 Hz, 2H, H-2′), 2.75 (dd, *J* = 4.7, 12.4 Hz, 1H, H-3e), 3.30 (dd, *J* = 7.1, 14.2 Hz, 1H, H-9a), 3.45 (dd, *J* = 1.7, 9.0 Hz, 1H, H-7), 3.53 (dd, *J* = 2.8, 14.1 Hz, 1H, H-9b), 3.68 (ddd, *J* = 2.9, 4.7, 11.8 Hz, 1H, H-4), 3.72-3.82 (m, 3H, H-5, H-6, H-8), 4.51, 4.70 (A, B of AB, *J* = 12.0 Hz, 2H, CH_2Ph), 7.38 (s, 5H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 12.1 (C-4′), 18.4 (C-3′), 22.6 (NHCO$\underline{C}$H$_3$), 37.0 (C-2′), 39.8 (C-3), 41.0 (C-9), 51.2 (C-5), 66.5 ($\underline{C}$H$_2$Ph), 67.6 (C-4), 68.9 (C-7), 69.3 (C-8), 71.9 (C-6), 100.4 (C-2), 127.6, 128.0, 128.0, 136.5 (6C, C_6H_5), 174.3, 176.7, 180.8 (3CO).

Sodium (benzyl 5-acetamido-3,5,9-trideoxy-9-hexanoylamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12f).
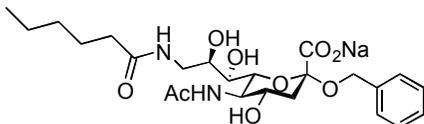
According to the general method II, compound **11f** (26 mg, 0.040 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12f** was obtained as a fluffy solid (20 mg, 96%).

$[\alpha]_D^{25}$ = -1.15 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.81 (t, *J* = 7.0 Hz, 3H, H-6′), 1.25 (m, 4H, H-4′, H-5'), 1.57 (m, 2H, H-3′), 1.66 (t, *J* = 12.2 Hz, 1H, H-3a), 2.02 (s, 3H, NHAc), 2.25 (t, *J* = 7.4 Hz, 2H, H-2′), 2.75 (dd, *J* = 4.7, 12.5 Hz, 1H, H-3e), 3.22 (dd, *J* = 7.7, 14.2 Hz, 1H, H-9a), 3.46 (dd, *J* = 1.7, 9.0 Hz, 1H, H-7), 3.58 (dd, *J* = 7.7, 14.1 Hz, 1H, H-9b), 3.68 (ddd, *J* = 6.6, 9.8, 12.8 Hz, 1H, H-4), 3.72-3.80 (m, 3H, H-5, H-6, H-8), 4.50, 4.70 (A, B of AB, *J* = 11.0 Hz, 2H, CH$_2$Ph), 7.40 (s, 5H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 20.1 (C-6′), 20.6 (C-5′), 21.8 (NHCO$\underline{C}$H$_3$), 23.7 (C-4′), 28.9 (C-3′), 34.2 (C-2′), 39.0 (C-3), 40.3 (C-9), 50.4 (C 5), 65.8 ($\underline{C}$H$_2$Ph), 66.8 (C-4), 68.2 (C-7), 68.7 (C-8), 71.1 (C-6), 99.6 (C-2), 126.8, 127.2, 127.3, 135.6 (6C, C$_6$H$_5$), 173.6, 176.1, 180.1 (3CO).

Sodium (benzyl 5-acetamido-9-decanoylamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12g).
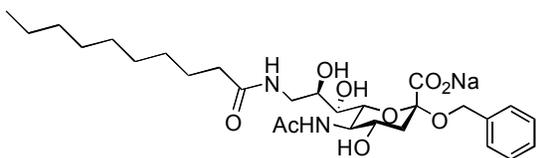
According to the general method II, compound **11g** (30 mg, 0.043 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12g** was obtained as a fluffy solid (24 mg, 97%).

$[\alpha]_D^{25}$ = - 4.1 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 0.56 (t, *J* = 6.8 Hz, 3H, H-10′), 0.92-1.00 (m, 12H, H-4′, H-5′, H-6′, H-7′, H-8′, H-9′), 1.32 (m, 2H, H-3′), 1.46 (t, *J* = 12.2 Hz, 1H, H-3a), 1.81 (s, 3H, NHAc), 2.01 (t, *J* = 7.2 Hz, 2H, H-2′), 2.55 (dd, *J* = 4.5, 12.2 Hz, 1H, H-3e), 2.94 (dd, *J* = 8.3, 14.1 Hz, 1H, H-9a), 3.25 (d, *J* = 11.1 Hz, 1H, H-7), 3.42-3.50 (m, 2H, H-4, H-9b), 3.54-3.63 (m, 3H, H-5, H-

6, H-8), 4.28, 4.49 (A, B of AB, *J* = 10.9 Hz, 2H, CH$_2$Ph), 7.10-7.17 (m, 5H, Ar-H); ^{13}C NMR (125 MHz, D$_2$O): δ 13.9 (C-10′), 22.4 (C-9′), 22.5 (NHCOC$\underline{C}$H$_3$), 25.9 (C-8′), 28.7 (C-7′), 29.0 (2C, C-5′, C-6′), 29.2 (C-4′), 31.6 (C-3′), 36.2 (C-2′), 41.0 (C-3), 42.4 (C-9), 52.2 (C-5), 68.0 ($\underline{C}$H$_2$Ph), 68.7 (C-4), 70.3 (C-7), 70.7 (C-8), 72.9 (C-6), 101.2 (C-2), 128.6, 129.0, 129.2, 137.4 (6C, C$_6$H$_5$), 173.6, 175.3, 177.3 (3CO).

Sodium [benzyl 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12h).
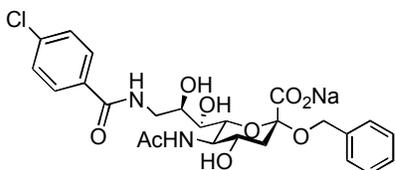
According to the general method II, compound **11h** (32.0 mg, 0.047 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12h** was obtained as a fluffy solid (21.0 mg, 75%).

$[\alpha]_D^{25}$ = - 1.95 (*c* = 1.00, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.68 (t, *J* = 12.2 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.76 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 3.45 (dd, *J* = 8.0, 14.4 Hz, 1H, H-9a), 3.51 (d, *J* = 8.9 Hz, 1H, H-7), 3.64-3.84 (m, 5H, H-4, H-5, H-6, H-8, H-9b) 4.51, 4.69 (A, B of AB, *J* = 11.2 Hz, 2H, CH$_2$Ph), 7.26-7.36, 7.46, 7.69 (m, 9H, Ar-H); ^{13}C NMR (125 MHz, D$_2$O): δ 22.3 (NHCOC$\underline{C}$H$_3$), 40.9 (C-3), 43.0 (C-9), 52.2 (C-5), 67.7 ($\underline{C}$H$_2$Ph), 68.6 (C-4), 70.3 (C-7), 70.6 (C-8), 73.0 (C-6), 101.6 (C-2), 128.5, 128.9, 129.0, 129.0, 129.1, 132.4, 137.6, 137.9 (12 C, C$_6$H$_5$, C$_6$H$_4$), 170.4, 173.5, 176.5 (3CO).

Sodium [benzyl 5-acetamido-3,5,9-trideoxy-9-(4-methoxybenzamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12i).
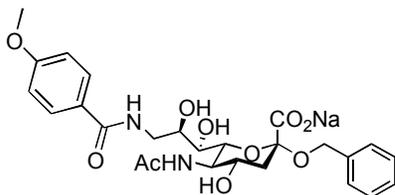
According to the general method II, compound **11i** (25.0 mg, 0.037 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12i** was obtained as a fluffy solid (16.0 mg, 80%).

$[\alpha]_D^{25}$ = - 3.22 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.68 (t, *J* = 12.2 Hz, 1H, H-3a), 1.98 (s, 3H, NHAc), 2.76 (dd, *J* = 4.7, 12.4 Hz, 1H, H-3e), 3.44 (dd, *J* = 8.2, 14.6 Hz, 1H, H-9a), 3.51 (dd, *J* = 1.7, 8.7 Hz, 1H, H-7), 3.64-3.84 (m, 8H, H-4, H-5, H-6, H-8, H-9b, OCH₃), 4.51, 4.68 (A, B of AB, *J* = 11.2 Hz, 2H, CH₂Ph), 7.01, 7.24-7.36, 7.74 (m, 9H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 22.4 (NHAc), 40.9 (C-3), 42.9 (C-9), 52.2 (C-5), 55.8 (PhOC̲H₃), 67.7 (C̲H₂Ph), 68.6 (C-4), 70.3 (C-7), 70.7 (C-8), 73.0 (C-6), 101.6 (C-2), 114.3, 126.3, 128.5, 128.9, 129.0, 129.5, 137.6, 162.3 (12 C, C₆H₅, C₆H₄), 170.6, 173.8, 175.4 (3CO).

Sodium [benzyl 5-acetamido-3,5,9-trideoxy-9-(4-methylbenzamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12j).
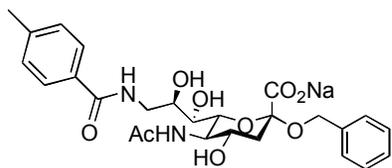
According to the general method II, compound **11j** (26.0 mg, 0.039 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12j** was obtained as a fluffy solid (20.0 mg, 87%).

$[\alpha]_D^{25}$ = - 2.07 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.68 (t, *J* = 12.2 Hz, 1H, H-3a), 1.98 (s, 3H, NHAc), 2.38 (s, 3H, PhC̲H₃), 2.76 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 3.33- 3.51 (m, 2H, H-7, H-9a), 3.67 (m, 1H, H-4), 3.72-3.83 (m, 4H, H-5, H-6, H-8, H-9b), 4.53, 4.69 (A, B of AB, *J* = 11.3 Hz, 2H, CH₂Ph), 7.26-7.36, 7.69 (m, 9H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 20.9 (PhC̲H₃), 22.3 (NHCOC̲H₃), 40.9 (C-3), 42.9 (C-9), 52.2 (C-5), 67.7 (C̲H₂Ph), 68.6 (C-4), 70.2 (C-7), 70.6 (C-8), 73.0 (C-6), 101.7 (C-2), 127.5, 128.5, 128.9, 129.0, 129.7, 131.0, 137.6, 143.6 (12C, C₆H₅, C₆H₄), 171.3, 173.8, 175.4 (3CO).

Sodium [benzyl 5-acetamido-9-(3,4-dichlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12k).
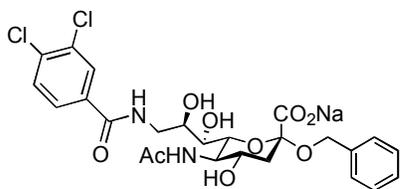
According to the general method II, compound **11k** (31.0 mg, 0.043 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12k** was obtained as a fluffy solid (20.0 mg, 75%).

$[\alpha]_D^{25}$ = - 2.45 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.68 (t, *J* = 12.1 Hz, 1H, H-3a), 2.01 (s, 3H, NHAc), 2.76 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 3.39 (dd, *J* = 14.4, 8.5, Hz, 1H, H-9a), 3.52 (m, 1H, H-7), 3.64-3.85 (m, 5H, H-4, H-5, H-6, H-8, H-9b), 4.47, 4.69 (A, B of AB, *J* = 11.1 Hz, 2H, CH_2Ph), 7.21-7.32, 7.43, 7.53, 7.75 (m, 8H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 22.4 (NHCO$\underline{C}$H$_3$), 41.0 (C-3), 43.2 (C-9), 52.2 (C-5), 67.6 ($\underline{C}$H$_2$Ph), 68.6 (C-4), 70.4 (C-7), 70.7 (C-8), 73.0 (C-6), 101.5 (C-2), 126.9, 128.5, 128.9, 129.4, 130.9, 132.5, 133.7, 135.8, 137.4 (12C, C_6H_5, C_6H_3), 168.5, 173.7, 175.4 (3CO).

Sodium [benzyl 5-acetamido-9-(3-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12l).
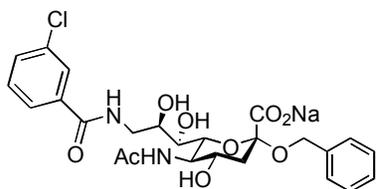
According to the general method II, compound **11l** (28.0 mg, 0.041 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12l** was obtained as a fluffy solid (16.0 mg, 69 %).

$[\alpha]_D^{25}$ = -1.77 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.69 (t, *J* = 11.8 Hz, 1H, H-3a), 2.00 (s, 3H, NHAc), 2.77 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 3.49 (m, 2H, H-7, H-9a), 3.63-3.83 (m, 5H, H-4, H-5, H-6, H-8, H-9), 4.53, 4.71 (A, B of AB, *J* = 11.2 Hz, 2H, CH_2Ph), 7.29-7.39, 7.44, 7.53, 7.62 (m, 9H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 21.9 (NHAc), 40.5 (C-3), 42.6 (C-9), 51.8 (C-5), 67.2 ($\underline{C}$H$_2$Ph), 68.2 (C-4), 69.8 (C-7), 70.1 (C-8), 72.6 (C-6), 101.2 (C-2), 125.3, 127.1, 128.1, 128.5,

128.5, 130.2, 131.8, 134.0, 135.3, 137.2 (12C, C_6H_5, C_6H_4), 169.5, 173.4, 175.0 (3CO).

Sodium [benzyl 5-acetamido-3,5,9-trideoxy-9-(4-trifluoromethylbenzamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12m).
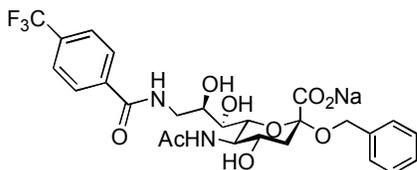
According to the general method II, compound **11m** (36.0 mg, 0.050 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12m** was obtained as a fluffy solid (21.0 mg, 70%).

$[\alpha]_D^{25}$ = -2.37 (c = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.68 (t, J = 12.2 Hz, 1H, H-3a), 2.00 (s, 3H, NHAc), 2.76 (dd, J = 4.7, 12.5 Hz, 1H, H-3e), 3.44-3.52 (m, 2H, H 7, H-9a), 3.68 (m, 1H, H-4), 3.75-3.84 (m, 4H, H-5, H-6, H-8, H-9b), 4.49, 4.68 (A, B of AB, J = 11.2 Hz, 2H, CH_2Ph), 7.24-7.35, 7.75, 7.84 (m, 9H, Ar-H); ^{13}C NMR (125 MHz, D_2O): δ 22.4 (NHCO$\underline{C}$H₃), 40.9 (C-3), 43.1 (C-9), 52.3 (C-5), 67.6 ($\underline{C}$H₂Ph), 68.6 (C-4), 70.3 (C-7), 70.6 (C-8), 73.0 (C-6), 101.6 (C-2), 126.0, ($\underline{C}$F₃), 128.0, 128.5, 128.9, 129.0, 129.5, 129.6, 132.5, 137.5 (12C, C_6H_5, C_6H_4), 170.0, 173.8, 175.4 (3CO).

Sodium [benzyl 5-acetamido-9-(2,4-dichorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (12n).
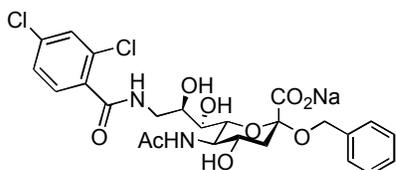
According to the general method II, compound **11n** (31.0 mg, 0.043 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup and purification **12n** was obtained as a fluffy solid (18.0 mg, 72%).

$[\alpha]_D^{25}$ = -1.17 (c = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.66 (t, J = 12.1 Hz, 1H, H-3a), 2.01 (s, 3H, NHAc), 2.75 (dd, J = 4.7, 12.3 Hz, 1H, H-3e), 3.53-3.83 (m, 6H, H-4, H-5, H-6, H-7, H-9), 3.89 (m, 1H, H-8), 4.49, 4.71 (A, B of AB, J = 11.0

Hz, 2H, CH$_2$Ph), 7.33-7.41, 7.55 (m, 8H, Ar-H); ^{13}C NMR (125 MHz, D$_2$O): δ 20.0 (NHCO$\underline{C}$H$_3$), 38.5 (C-3), 40.1 (C-9), 49.9 (C-5), 65.1 ($\underline{C}$H$_2$Ph), 66.1 (C-4), 67.4 (C-7), 67.9 (C-8), 70.5 (C-6), 98.9 (C-2), 125.4, 126.2, 126.6, 126.7, 127.1, 127.4, 127.6, 129.0, 131.3, 134.1, 135.0 (12C, C$_6$H$_5$, C$_6$H$_3$), 167.6, 171.4, 173.0 (3XCO).

Benzyl 5-acetamido-3,5,9-trideoxy-9-(4-nitrobenzamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranoside (12o).
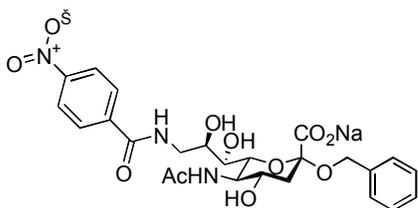
According to the general method II, compound **11o** (28.0 mg, 0.040 mmol) was deprotected by treatment with 10% NaOH (0.2 mL). After the workup (this compound was not passed through the Dowex 50X8 (Na) column) and purification **12o** was obtained as a fluffy solid (21.0 mg, 95%).

$[\alpha]_D^{25}$ = -2.45 (*c* = 1.00, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.67 (t, *J* = 12.2 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.74 (dd, *J* = 4.7, 12.4 Hz, 1H, H-3e), 3.46 (dd, *J* = 7.7, 14.0 Hz, 1H, H-9a), 3.52 (dd, *J* = 1.7, 8.8 Hz, 1H, H-7), 3.67 (m, 1H, H-4), 3.74-3.84 (m, 4H, H-5, H-6, H-8, H-9b), 4.48, 4.67 (A, B of AB, *J* = 11.2 Hz, 2H, CH$_2$Ph), 7.23-7.34, 7.88, 8.25 (m, 9H, Ar-H); ^{13}C NMR (125 MHz, D$_2$O): δ 20.4 (NHCO$\underline{C}$H$_3$), 39.0 (C-3), 41.3 (C-9), 50.3 (C-5), 65.7 ($\underline{C}$H$_2$Ph), 66.6 (C-4), 68.4 (C-7), 68.6 (C-8), 71.1 (C-6), 99.6 (C-2), 122.3, 126.6, 126.8, 127.0, 127.0, 135.6, 138.1, 147.8 (12C, Ar-C), 172.4, 173.5, 176.5 (3CO).

benzyl 5-acetamido-9-{4-methyl-(1*H*-benzotriazole)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (13a)
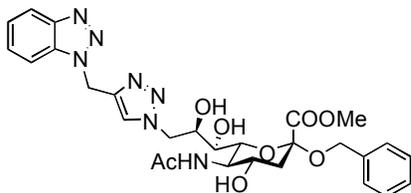
To solution of compound **8** (25 mg, 57.0 μmol) and 1-Propargyl-1*H*-benzotriazole (9.00 mg, 57.0 μmol) in tBuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.20 mg, 6.00 μmol, 6 μl of freshly prepared solution of 1M in H$_2$O), followed by CuSO$_4$.5H$_2$O (0.15 mg, 0.61 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which at intervals was added the 2 portions of Na-ascorbate (2.20 mg, 12.0 μmol) and 2 portions of CuSO$_4$.5H$_2$O (0.30 mg, 1.20 μmol). After 36 h TLC in DCM:MeOH (7:3) system showed completion of reaction. Solvents removed under high vaccum to dryness. This was purified by column chromatography on silica gel in DCM:MeOH system to get a foamy solid of **13a** (25.0 mg, 75%).

^{1}H NMR (500 MHz, CD$_3$OD): δ 1.79 (t, *J* = 12.7 Hz, 1H, H-3a), 1.94 (s, 3H, NHAc), 2.70 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.37 (dd, *J* = 1.5, 8.5 Hz, 1H, H-7), 3.55-3.68 (m, 2H, H-4, H-6), 3.72 (s, 3H, CO$_2$CH$_3$), 3.77 (q, *J* = 10.2 Hz, 1H, H-5), 4.10 (m, 1H, H-8), 4.42 (dd, *J* = 4.2, 8.0 Hz, 1H, H-9a), 4.46, 4.74 (A, B of AB, *J* = 11.8 Hz, 2H, CH$_2$Ph), 4.81 (dd, *J* = 2.5, 4.1 Hz, 1H, H-9b), 6.04 (s, 2H, -CH$_2$-N), 7.19-7.28 (m, 5H, Ar-H), 7.40, 7.53, 7.85, 7.96 (m, 4H, Ar-H), 7.98 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 23.0 (NHCOCH$_3$), 42.1 (C-3), 44.6 (-CH$_2$-N) 53.7 (CO$_2$CH$_3$), 54.2 (C-5), 55.3 (C-9), 67.8 (CH$_2$Ph), 68.7 (C-4), 71.5 (C-8), 71.9 (C-7), 75.2 (C-6), 100.7 (C-2), 112.12, 120.37, 126.1, 127.0, 129.1, 129.2, 129.3, 129.6, 130.3, 134.6, 139.3, 143.1, 147.2 (14C, Ar-C), 171.2, 175.6 (2CO).

Methyl [benzyl 5-acetamido-9-{4-(3-pyridyl)1,2,3-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (13b)
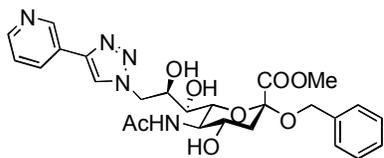
To solution of compound **8** (25 mg, 57.0 μmol) and 3-ethynyl pyridine (5.78 mg, 57.0 μmol) in tBuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.20 mg, 6.00 μmol, 6 μl of freshly prepared solution of 1M in H$_2$O), followed by CuSO$_4$.5H$_2$O (0.15 mg, 0.61 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which at intervals was added the 2 portions of Na-ascorbate (2.20 mg,

12.0 μmol) and 2 portions of CuSO$_4$·5H$_2$O (0.30 mg, 1.20 μmol). After 36 h, according to TLC (EA:MeOH 7:3), reaction was complete. Solvents removed under high vaccum to dryness and the residue purified by column chromatography on silica gel (EA:MeOH) to get **13b** (23 mg, 74%) as a foam.

^{1}H NMR (500 MHz, CD$_3$OD): δ 1.73 (t, *J* = 12.4 Hz, 1H, H-3a), 1.88 (s, 3H, NHAc), 2.64 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.35 (dd, *J* = 1.4, 8.5 Hz, 1H, H-7), 3.59 (m, 2H, H-4, H-6), 3.64 (s, 3H, CO$_2$CH$_3$), 3.72 (q, *J* = 10.2 Hz, 1H, H-5), 4.15 (m, 1H, H-8), 4.44 (m, 2H, H-9a, A of AB CH$_2$Ph), 4.72 (B of AB, *J* = 11.7 Hz, 2H, CH$_2$Ph), 4.83 (dd, *J* = 2.7, 14.0 Hz, 1H, H-9b), 7.42, 7.12-7.20, 8.92, 8.39, 8.18 (m, 10H, Ar-H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 23.0 (NHCOC̲H$_3$), 42.2 (C-3), 53.7 (CO$_2$C̲H$_3$), 54.3 (C-5), 55.5 (C-9), 67.8 (C̲H$_2$Ph), 68.7 (C-4), 71.5 (C-8), 72.0 (C-7), 75.3 (C-6), 100.7 (C-2), 124.9, 126.0, 129.1, 129.3, 129.7, 135.3, 139.3, 145.4, 147.6, 149.9 (13C, Ar-C), 171.3, 175.6 (2CO).

Methyl [benzyl 5-acetamido-9-(4-methylphenoxy)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (13c)
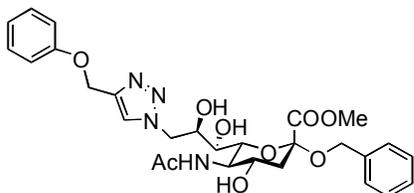
To solution of **8** (25 mg, 57.0 μmol) and Phenyl propargyl ether (7.52 mg, 57.0 μmol) in *tert*BuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.20 mg, 6.00 μmol, 6 μl of freshly prepared solution of 1M in H$_2$O), followed by CuSO$_4$·5H$_2$O (0.15 mg, 0.61 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which at intervals was added the 2 portions of Na-ascorbate (2.20 mg, 12.0 μmol) and 2 portions of CuSO$_4$.5H$_2$O (0.30 mg, 1.20 μmol). After 36 h, according to TLC (EA:MeOH 7:3), reaction was complete. Solvents removed under high vaccum to dryness and the residue purified by column chromatography on silica gel (EA:MeOH) to get **13c** (26 mg, 81%) as a solid.

^{1}H NMR (500 MHz, CD$_3$OD): δ 1.82 (t, *J* = 12.4 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.73 (dd, *J* = 4.7, 12.7 Hz, 1H, H-3e), 3.41 (dd, *J* = 1.4, 8.4 Hz, 1H, H-7), 3.62-3.68 (m, 2H, H-4, H-6), 3.75 (s, 3H, CO$_2$CH$_3$), 3.80 (q, *J* = 10.2 Hz, 1H, H-

5), 4.17 (m, 1H, H-8), 4.45-4.53 (m, 2H, H-9a, A of AB CH$_2$Ph), 4.80 (B of AB, J = 11.7 Hz, 2H, CH$_2$Ph), 4.84 (m, 1H, H-9b), 5.16 (s, 2H, -CH$_2$-OPh), 6.92-7.01 (m, 3H, Ar-H), 7.22-7.33 (m, 7H, Ar-H), 8.05 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 23.0 (NHCO<u>C</u>H$_3$), 42.1 (C-3), 53.7 (CO$_2$<u>C</u>H$_3$), 54.2 (C-5), 55.3 (C-9), 62.7 (-<u>C</u>H$_2$-OPh), 67.8 (<u>C</u>H$_2$Ph), 68.7 (C-4), 71.5 (C-8), 71.9 (C-7), 75.2 (C-6), 100.7 (C-2), 116.2, 122.6, 127.0, 129.1, 129.2, 129.6, 130.9, 131.6, 139.3, 145.1, (14C, Ar-C), 171.3, 175.6 (2CO).

Methyl [benzyl 5-acetamido-9-{4-(3-thiophene)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (SH-13d)
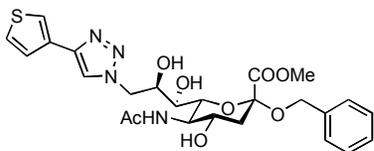
To solution of **8** (25 mg, 57.0 μmol) and 3-ethynyl thiophene (6.15 mg, 57.0 μmol) in tBuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.20 mg, 6.00 μmol, 6 μl of freshly prepared solution of 1M in H$_2$O), followed by CuSO$_4$.5H$_2$O (0.15 mg, 0.61 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which at intervals was added the two additional portions of Na-ascorbate (2.20 mg, 12.0 μmol) and two additional portions of CuSO$_4$.5H$_2$O (0.30 mg, 1.20 μmol). Reaction was very sluggish as monitored by TLC (EA:MeOH 8:2). Solvents removed under high vaccum to dryness and the residue purified by column chromatography on silica gel (EA:MeOH) to get **13d** (10 mg, 32%) as a solid.

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.95 (s, 3H, NHAc), 2.02 (m, 1H, H-3a), 2.82 (dd, J = 4.6, 13.2 Hz, 1H, H-3e), 3.31 (d, J = 9.0 Hz, 1H, H-7), 3.45 (d, J = 10.4 Hz, 1H, H-6), 3.66 (s, 3H, CO$_2$CH$_3$), 3.72 (m, 1H, H-4), 3.84 (m, 1H, H-5), 4.20 (m, 1H, H-8), 4.41, 4.75 (A, B of AB, J = 11.7 Hz, 2H, CH$_2$Ph), 4.53 (dd, J = 7.0, 14.1 Hz, 1H, H-9a), 4.83 (dd, J = 2.0, 14.1 Hz, 1H, H-9b), 7.23-7.43 (m, 7H, Ar-H), 7.66, 7.92 (s, 2H, Ar-H). ^{13}C NMR (125 MHz, CDCl$_3$): δ 23.3 (NHCO<u>C</u>H$_3$), 40.5 (C-3), 53.6 (C-5), 53.7 (2C, C-9, CO$_2$<u>C</u>H$_3$), 67.7 (<u>C</u>H$_2$Ph), 67.7 (C-4), 70.1 (C-8), 70.4 (C-7), 74.1 (C-6), 99.2 (C-2), 121.7, 126.1, 127.0, 128.0, 128.2, 128.7, 137.2 (11C, Ar-C), 170.5, 174.5 (2CO).

Methyl [benzyl 5-acetamido-9-{4-(3-chloro phenyl)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (13e)
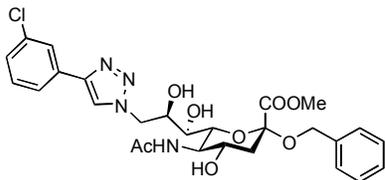
To solution of **8** (25 mg, 57.0 μmol) and 3-chloro-1-ethynyl benzene (7.75 mg, 57.0 μmol) in tBuOH:H_2O (1:1) (0.5 ml) was added Na-ascorbate (1.20 mg, 6.00 μmol, 6 μl of freshly prepared solution of 1M in H_2O), followed by $CuSO_4.5H_2O$ (0.15 mg, 0.61 μmol) in H_2O. This heterogeneous mixture was stirred for 36 h during which at intervals was added the two additional portions of Na-ascorbate (2.20 mg, 12.0 μmol) and two additional portions of $CuSO_4·5H_2O$ (0.30 mg, 1.20 μmol). Reaction was sluggish as monitored by TLC (EA:MeOH 8:2). Solvents removed under high vaccum to dryness and the residue purified by column chromatography on silica gel (EA:MeOH) to get **13e** (18 mg, 56%) as a solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.97 (m, 3H, H-3a, NHAc), 2.83 (dd, *J* = 4.2, 12.9 Hz, 1H, H-3e), 3.32 (d, *J* = 8.5 Hz, 1H, H-7), 3.47 (m, 1H, H-6), 3.67 (s, 3H, CO_2CH_3), 3.72 (m, 1H, H-4), 3.82 (q, *J* = 9.4 Hz, 1H, H-5), 4.19 (m, 1H, H-8), 4.41, 4.73 (A, B of AB, *J* = 11.7 Hz, 2H, CH_2Ph), 4.53 (dd, *J* = 6.8, 14.0 Hz, 1H, H-9b), 4.84 (d, *J* = 13.5 Hz, 1H, H-9b), 6.86 (d, J = 7.2 Hz, 1H, NH-5), 7.22-7.34, 7.67 (m, 8H, Ar-H), 7.70, 7.96 (s, 2H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.1 (NHCO$\underline{C}H_3$), 40.8 (C-3), 53.4 (C-5), 53.7 (C-9, $CO_2\underline{C}H_3$), 66.8 ($\underline{C}H_2Ph$), 67.8 (C-4), 70.0 (C-8), 70.4 (C-7), 74.1 (C-6), 99.2 (C-2), 122.6, 124.1, 126.1, 128.1, 128.3, 128.6, 128.7, 130.6, 132.5, 137.1, 146.7 (14C, Ar-C), 170.5, 174.6 (2CO).

Methyl [benzyl 5-acetamido-9-{4-(4-trifluormethyl phenyl)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (13f).
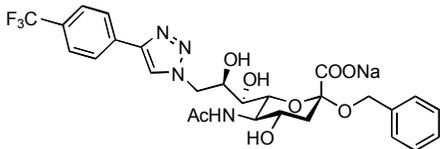
To solution of **8** (25 mg, 57.0 μmol) and 4-trifluoromethyl benzene acetylene (9.69 mg, 57.0 μmol) in tBuOH:H_2O (1:1) (0.5 ml) was added Na-ascorbate (1.20

mg, 6.00 μmol, 6 μl of freshly prepared solution of 1M in H_2O), followed by $CuSO_4.5H_2O$ (0.15 mg, 0.60 μmol) in H_2O. This heterogeneous mixture was stirred for 36 h during which at intervals was added two additional portions of Na-ascorbate (2.20 mg, 12.0 μmol) and two additional portions of $CuSO_4·5H_2O$ (0.30 mg, 1.20 μmol). After 36 h, according to TLC (EA:PE:MeOH 7:2:1), reaction was complete. Solvents removed under high vacuum to dryness and the residue purified by column chromatography on silica gel to get **13f** (22 mg, 64%) as solid. ^1H NMR (500 MHz, CD_3OD): δ 1.82 (t, J = 12.4 Hz, 1H, H-3a), 1.97 (s, 3H, NHAc), 2.73 (dd, J = 4.7, 12.8 Hz, 1H, H-3e), 3.43 (dd, J = 1.4, 8.5 Hz, 1H, H-7), 3.68 (m, 2H, H-4, H-6), 3.73 (s, 3H, CO_2CH_3), 3.80 (q, J = 10.2 Hz, 1H, H-5), 4.23 (m, 1H, H-8), 4.53 (m, 2H, H-9a, B of AB CH_2Ph), 4.81 (d, J = 11.7 Hz, 2H, B of AB CH_2Ph), 4.90 (m, 1H, H-9b), 7.25 (m, 5H, Ar-H), 7.72 (d, J = 8.2 Hz, 2H, Ar-H), 8.01 (d, J = 8.1 Hz, 2H, Ar-H), 8.43 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, CD_3OD): δ 23.0 (NHCO$\underline{C}H_3$), 42.2 (C-3), 53.7 (CO$_2\underline{C}H_3$), 54.2 (C-5), 55.4 (C-9), 67.8 ($\underline{C}H_2Ph$), 68.7 (C-4), 71.4 (C-8), 71.9 (C-7), 75.3 (C-6), 100.7 (C-2), 125.0, 127.3, 127.4, 129.1, 129.2, 129.6, 136.1, 139.3, 147.4 (12C, Ar-C), 171.3, 175.6 (2CO).

Sodium [benzyl 5-acetamido-9-{4-methyl (1-H-benzotriazole)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (14a)
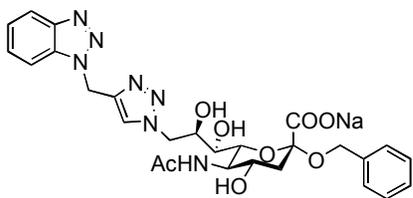
To a solution of compound **13a** (25 mg, 0.042 mmol) in methanol (2 mL) was added 10% aq. NaOH (0.2 mL). The mixture was stirred at r.t. for 3 h. After neutralization with 10 % HCl till pH=7, the solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na^+ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **14a** (25 mg, 98%) as a white solid after a final lyophilization from water.

^{1}H NMR (500 MHz, D$_2$O): δ 1.44 (t, J = 12.7 Hz, 1H, H-3a), 1.92 (s, 3H, NHAc), 2.56 (dd, J = 4.5, 12.4 Hz, 1H, H-3e), 3.45 (dd, J = 1.2, 9.1 Hz, 1H, H-7), 3.30-3.51 (m, 6H, H-4, H-5, H-6, H-8, A, B of AB CH$_2$Ph), 4.32 (dd, J = 9.5, 14.4 Hz, 1H, H-9a), 4.69-4.81 (m, 1H, H-9b), 5.59 (q, J = 16.0 Hz, 2H, -CH$_2$-N), 6.33 (t, J = 7.5 Hz, 1H, Ar-H), 6.61 (t J = 7.4 Hz, 2H, Ar-H), 6.80 (m, 2H, Ar-H), 6.88 (t, J = 7.4 Hz, Ar-H), 6.86 (t, J = 7.3 Hz, 1H, Ar-H), 7.18 (d, J = 8.3 Hz, 1H, Ar-H), 8.21 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 22.3 (NHCOCH$_3$), 40.9 (C-3), 42.7 (-CH$_2$-N), 52.1 (C-5), 53.7 (C-9), 67.3 (CH$_2$Ph), 68.4 (C-4), 69.9 (C-7), 1.5 (C-8), 72.7 (C-6), 101.3 (C-2), 110.1, 118.0, 124.5, 126.5, 128.0, 128.1, 128.2, 128.9, 132.1, 136.4, 141.9, 144.1 (14C, Ar-C), 173.3, 175.4 (2CO).

Sodium [benzyl 5-acetamido-9-{4-(3-pyridyl) triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (14b)
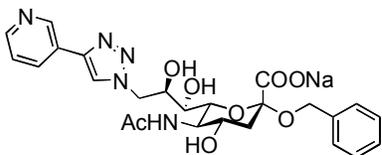
To a solution of compound **13b** (25 mg, 0.042 mmol) in methanol (2 mL) was added 10% aq. NaOH (0.2 mL). The mixture was stirred at r.t. for 3 h. After neutralization with 10 % HCl till pH=7, the solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na$^+$ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **14b** (23 mg, 98%) as a white solid after a final lyophilization from water.

^{1}H NMR (500 MHz, CD$_3$OD): δ 1.69 (t, J = 12.2 Hz, 1H, H-3a), 2.00 (s, 3H, NHAc), 2.75 (dd, J = 4.6, 12.4 Hz, 1H, H-3e), 3.46 (dd, J = 1.6, 9.0 Hz, 1H, H-7), 3.68 (m, 1H, H-4), 3.74 (dd, J = 1.7, 10.4 Hz, 1H, H-6), 3.80 (m, 2H, H-5, H-8), 4.44 (m, 2H, H-9a, A of AB CH$_2$Ph), 4.57 (B of AB, J = 11.7 Hz, 1H, CH$_2$Ph), 4.75 (m, 1H, H-9b), 7.10-7.26, 7.37(m, 6H, Ar-H), 7.84 (d, J = 8.0 Hz, Ar-H), 8.22 (d, J = 4.0 Hz, 1H, Ar-H), 8.54 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 21.8 (NHCOCH$_3$), 40.5 (C-3), 51.7 (C-5), 52.9 (C-9), 7.3 (CH$_2$Ph), 68.0 (C-4), 69.2 (C-7), 70.3 (C-8), 72.3 (C-6), 101.3 (C-2), 111.9, 123.3, 124.3, 125.9, 127.8, 128.1, 128.2, 133.8, 137.1, 143.8, 145.2, 148.0 (13C, Ar-C), 171.3, 175.6 (2CO).

125

Methyl [benzyl 5-acetamido-9-{4-(3-thiophene)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (14c)
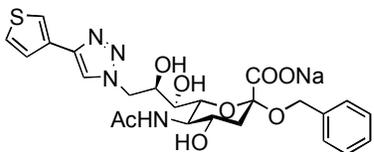
To a solution of compound **13c** (10 mg, 0.018 mmol) in methanol (2 mL) was added 10% aq. NaOH (0.2 mL). The mixture was stirred at r.t. for 3 h. After neutralization with 10 % HCl till pH=7, the solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na$^+$ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **14c** (7.50 mg, 75%) as a white solid after a final lyophilization from water.

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.69 (m, 1H, H-3a), 1.93 (s, 3H, NHAc), 2.75 (dd, *J* = 4.6, 12.5 Hz, 1H, H-3e), 3.38 (d, *J* = 1.8, 9.0 Hz, 1H, H-7), 3.62-3.71 (m, 3H, H-4, H-6, H-8), 3.78 (m, 1H, H-5), 4.44 (dd, *J* = 7.9, 14.5 Hz, 1H, H-9a), 4.55, 4.62 (d, *J* = 11.8 Hz, 2H, A, B of AB, CH$_2$Ph), 4.71 (m, 1H, H-9b), 7.18-7.25 (m, 3H, Ar-H), 7.33 (m, 2H, Ar-H), 7.52, 7.57, 7.81 (m, 3H, Ar-H), 8.32 (s, 1H, Ar-H).

Sodium [benzyl 5-acetamido-9-{4-(methylphenoxy)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (14d)
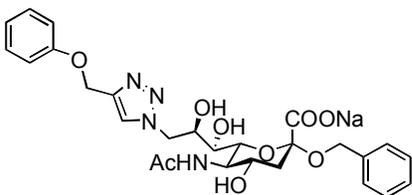
To a solution of compound **13d** (26 mg, 0.045 mmol) in methanol (2 mL) was added 10% aq. NaOH (0.2 mL). The mixture was stirred at r.t. for 3 h. After neutralization with 10 % HCl till pH=7, the solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na$^+$ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **14d** (26 mg, 98%) as a white solid after a final lyophilization from water.

^{1}H NMR (500 MHz, D$_2$O): δ 1.67 (t, J = 12.7 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.75 (dd, J = 4.6, 12.4 Hz, 1H, H-3e), 3.38 (dd, J = 1.8, 9.0 Hz, 1H, H-7), 3.65 (m, 3H, H-4, H-6, H-8), 3.77 (m, 1H, H-5), 4.37 (dd, J = 8.5, 14.5 Hz, 1H, H-9a), 4.53 (q, J = 11.7 Hz, 2H, A, B of AB CH$_2$Ph), 4.68 (dd, J = 2.2, 14.5 Hz, 1H, H-9b), 5.24 (s, 2H, -CH$_2$-O-Ph), 6.33 (t, J = 7.5 Hz, 1H, Ar-H), 7.01 (m, 3H, Ar-H), 7.20 (m, 3H, Ar-H), 7.30 (m, 4H, Ar-H), 8.05 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 19.9 (NHCOCH$_3$), 38.6 (C-3), 49.7 (C-5), 50.8 (C-9), 59.0 (-CH$_2$-O-Ph), 65.5 (CH$_2$Ph), 66.1 (C-4), 67.3 (C-7), 68.3 (C-8), 70.4 (C-6), 99.5 (C-2), 113.4, 120.0, 124.0, 126.0, 126.3, 126.4, 127.8, 135.4, 141.2, 155.1 (14C, Ar-C), 171.2, 173.0 (2CO).

Sodium [benzyl 5-acetamido-9-{4-(3-chloro phenyl)[1,2,3]-triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (14e)
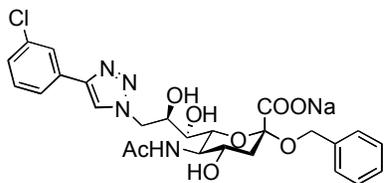
To a solution of compound **13e** (18 mg, 0.031 mmol) in methanol (2 mL) was added 10% aq. NaOH (0.2 mL). The mixture was stirred at r.t. for 3 h. After neutralization with 10 % HCl till pH=7, the solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na$^+$ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **14e** (12 mg, 99%) as a white solid after a final lyophilization from water.

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.68 (t, J = 12.1 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.75 (dd, J = 4.6, 12.4 Hz, 1H, H-3e), 3.44 (d, J = 1.8, 9.0 Hz, 1H, H-7), 3.67 (m, 1H, H-4), 3.72-3.82 (m, 3H, H-5, H-6, H-8), 4.41 (dd, J = 8.3, 14.5 Hz, 1H, H-9a), 4.48, 4.60 (d, J = 11.6 Hz, 2H, A, B of AB, CH$_2$Ph), 4.74 (m, 1H, H-9b), 7.12-7.52, 7.55 (m, 9H, Ar-H), 8.16 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 22.2 (NHCOCH$_3$), 40.9 (C-3), 52.1 (C-5), 53.4 (C-9), 67.8 (CH$_2$Ph), 68.4 (C-4), 69.7 (C-7), 70.8 (C-8), 72.7 (C-6), 99.2 (C-2), 123.5, 124.0, 125.4, 128.2, 128.5, 128.6, 128.7, 130.7, 131.4, 134.4, 137.6, 146.3 (13C, Ar-C), 173.5, 175.4 (2CO).

Sodium [benzyl 5-acetamido-9-{4-(4-trifluormethyl phenyl) triazole}-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (14f).
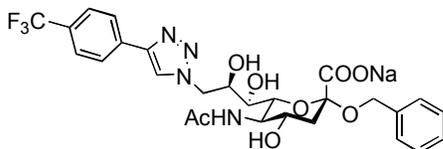
To a solution of compound **13f** (22 mg, 0.036 mmol) in methanol (2 mL) was added 10% aq. NaOH (0.2 mL). The mixture was stirred at r.t. for 3 h. After neutralization with 10 % HCl till pH=7, the solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na$^+$ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **14f** (16 mg, 73%) as a white solid after a final lyophilization from water.

^{1}H NMR (500 MHz, CD$_3$OD): δ 1.68 (t, J = 12.1 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.74 (dd, J = 4.6, 12.4 Hz, 1H, H-3e), 3.44 (dd, J = 1.8, 9.0 Hz, 1H, H-7), 3.63 (m, 1H, H-4) 3.73 (dd, J = 1.9, 10.5 Hz, 1H, H-6), 3.78 (m, 2H, H-5, H-8), 4.39 (dd, J = 8.4, 14.6 Hz, 1H, H-9a) 4.45 (d, J = 11.5 Hz, 1H, A of AB CH$_2$Ph), 4.58 (d, J = 11.6 Hz, 1H, B of AB CH$_2$Ph), 4.71 (d, J = 2.0, 14.6 Hz, 1H, H-9b), 7.10-7.18 (m, 3H, Ar-H), 7.24 (m, 2H, Ar-H), 7.54 (d, J = 8.2 Hz, 2H, Ar-H), 7.63 (d, J = 8.3 Hz, 2H, Ar-H), 8.22 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 20.0 (NHCO$\underline{C}$H$_3$), 38.7 (C-3), 49.9 (C-5), 51.1 (C-9), 65.6 ($\underline{C}$H$_2$Ph), 66.2 (C-4), 67.5 (C-7), 68.6 (C-8), 70.5 (C-6), 99.5 (C-2), 121.6, 123.7, 123.8, 126.0, 126.4, 126.5, 130.9, 135.3, 144 (14C, Ar-C), 171.3, 173.2 (2CO).

Methyl (methyl 5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate. (15)
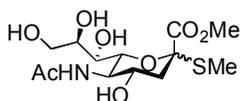
A solution of **3** (6.00 g, 11.5 mmol) in dry methanol (220 mL) was treated with 1 M methanolic NaOMe (24.0 mL) at r.t. for 2 h. The reaction mixture was neutralized with Amberlyst 15 (H$^+$) ion-exchange resin and filtered through a pad of Celite. The Celite was washed thoroughly with methanol (3 × 5 mL), and the

combined filtrates were evaporated to give **15** as anomeric mixture (α:β X:X) (2.70 g, 68%).

β-isomer:

^{1}H NMR (500MHz, CD$_3$OD): δ 1.92 (t, *J* = 13.8 Hz, 1H, H-3a), 2.04 (s, 3H, NHAc), 2.02 (s, 3H, SMe), 2.42 (dd, *J* = 4.9, 13.8 Hz, 1H, H-3e), 3.49 (dd, *J* = 1.0, 9.4 Hz, 1H, H-7), 3.63 (dd, *J* = 4.9, 11.5 Hz, 1H, H-9a), 3.78 (m, 6H, H-5, H-8, H-9b, CO$_2$CH$_3$), 4.05 (m, H-4), 4.10 (dd, *J* = 1.2, 10.7 Hz, H-6). ^{13}C NMR (125 MHz, CD$_3$OD): δ 12.9 (SCH$_3$), 23.8 (NHCOCH$_3$), 42.1 (C-3), 53.9 (CO$_2$CH$_3$), 54.7 (C-5), 66.2 (C-9), 69.3 (C-4), 71.2 (C-7), 72.3 (C-8), 73.6 (C-6), 86.5 (C-2), 172.0, 176.2 (2CO). **α-Isomer:** ^{1}H NMR (500MHz, CD$_3$OD): δ 1.76 (t, *J* = 12.8 Hz, 1H, H-3a), 1.98 (s, 3H, NHAc), 2.13 (s, 3H, SMe), 2.73 (dd, *J* = 4.7, 12.8 Hz, 1H, H-3e), 3.40 (dd, *J* = 1.6, 10.4 Hz, H-6), 3.49 (dd, *J* = 1.6, 9.1 Hz, 1H, H-7), 3.59-3.67 (m, 2H, H-4, H-9a), 3.74-3.84 (m, 6H, H-5, H-8, H-9b, CO$_2$CH$_3$). ^{13}C NMR (125 MHz, CD$_3$OD): δ 12.3 (SCH$_3$), 23.1 (NHCOCH$_3$), 42.2 (C-3), 54.0 (CO$_2$CH$_3$), 54.1 (C-5), 64.9 (C-9), 69.3 (C-4), 71.6 (C-7), 73.0 (C-8), 77.4 (C-6), 84.0 (C-2), 172.2, 175.7 (2CO).

Methyl [methyl 5-acetamido-3,5-dideoxy-9-*O*-(4-toluenesulfonyl)-2-thio-D-*glycero*-D-*galacto*-2-nonulo-pyranosid]onate: (16)
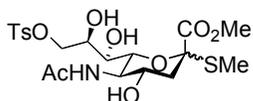
To a anomeric mixture of **15** (2.70 g, 7.64 mmol) in pyridine (80 ml) was added *p*-TsCl (1.45 gm, 7.64 mmol) at 0°C. After 2 h, *p*-TsCl (725 mg, 3.82 mmol) was added and stirring continued for 16 h at 5°C. The reaction mixture was warmed to r.t., diluted with methanol (40 mL) and stirring continued for 30 min. After removal of the solvents the remaining syrup was purified by chromatography on silica gel (DCM/MeOH 19:1) to yield **16** as anomeric mixture (α:β X:X) (2.13 gm, 56%).

β-Isomer: ^{1}H NMR (500MHz, CD$_3$OD): δ 1.90 (t, *J* = 13.9 Hz, 1H, H-3a), 1.97 (s, 3H, NHAc), 1.99 (s, 3H, SMe), 2.41 (m, 1H, H-3e), 2.43 (s, 3H, PhCH$_3$), 3.45 (dd, *J* = 1.1, 9.1 Hz, 1H, H-7), 3.71 (t, *J* = 10.4 Hz, 1H, H-5), 3.75 (s, 3H, CO$_2$CH$_3$),

3.95 (m, 1H, H-8), 4.01-4.07 (m, 3H, H-4, H-6, H-9a), 4.28 (dd, J = 2.2, 10.0 Hz, 1H, H-9b), 7.43 (d, J = 8.1 Hz, 2H, Ar-H), 7.79 (d, J = 8.4 Hz, 2H, Ar-H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 11.6 (S$\underline{C}$H$_3$), 22.0 (Ph$\underline{C}$H$_3$), 23.1 (NHCO$\underline{C}$H$_3$), 41.4 (C-3), 53.6 (CO$_2$$\underline{C}H_3$), 54.4 (C-5), 68.4 (C-4), 69.3 (C-8), 70.2 (C-7), 72.7 (C-6), 74.5 (C-9), 85.9 (C-2), 129.5, 131.5, 134.5, 146.9 (Ar-C), 171.2, 175.6 (2CO). **α-Isomer:** ^{1}H NMR (500MHz, CD$_3$OD): δ 1.72 (t, J = 12.8 Hz, 1H, H-3a), 1.97 (s, 3H, NHAc), 2.07 (s, 3H, SMe), 2.43 (s, 3H, PhCH$_3$), 2.71 (dd, J = 4.7, 12.9 Hz, 1H, H-3e), 3.36 (dd, J = 1.4, 10.3 Hz, H-6), 3.41 (dd, J = 1.4, 8.8 Hz, 1H, H-7), 3.62 (m, 1H, H-4), 3.70 (t, J = 10.1 Hz, 1H, H-5), 3.79 (s, 3H, CO$_2$CH$_3$), 3.96 (m, 1H, H-8), 4.05 (dd, J = 6.1, 10.2 Hz, 1H, H-9a), 4.28 (dd, J = 2.0, 10.2 Hz, 1H, H-9b), 7.43 (d, J = 8.2 Hz, 2H, Ar-H), 7.82 (d, J = 8.3 Hz, 2H, Ar-H). ^{13}C NMR (125 MHz, CD$_3$OD): δ 12.2 (S$\underline{C}$H$_3$), 21.8 (PhCH$_3$), 22.9 (NHCO$\underline{C}$H$_3$), 42.0 (C-3), 53.9 (CO$_2$CH$_3$), 54.0 (C-5), 69.0 (C-4), 70.2 (C-7), 70.3 (C-8), 73.7 (C-6), 84.0 (C-2), 129.3, 131.3, 134.4, 146.7 (Ar-C), 171.7, 175.6 (2CO).

Methyl (methyl 5-acetamido-9-azido-2-thio-3,5,9-trideoxy-D-*glycero*-D-*galacto*-2-nonulo-pyranosid)onate: (17)

A mixture of **SH-16** (2.13 gm, 4.21 mmol), crown ether 18-C-6 (447 mg, 1.68 mmol) and NaN$_3$ (1.37 gm, 21.1 mmol) was stirred in DMF (60 mL) at 60°C for 24 h. TLC analysis (DCM/acetone 1:1) showed completion of the reaction. The mixture was filtered through a pad of Celite and then washed with methanol several times. The filtrate was collected and evaporated to dryness. Purification by column chromatography on silica gel (DCM/acetone 7:3) yielded **17** as a mixture (1.42 gm, 89%) of α:β isomer (0.8:1).

Methyl (methyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-2-thio-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate: (18)

Compound **17** (1.42 gm, 3.75 mmol) was dissolved in dry pyridine (1.60 gm, 157 mmol) and stirred at 0°C for 15 min under argon before DMAP (73.0 mg, 0.60 mmol) and Ac$_2$O (1.47 gm, 187 mmol) were added simultaneously at 0°C. After stirring at r.t. for 14 h, TLC analysis showed completion of the reaction. After evaporation of the solvents, chromatography on silica gel (DCM/MeOH 20:1) yielded **18** as a white foam (1.69 gm, 90%) with a mixture of α:β isomer (0.8:1).

Methyl [(3-NHCbz-propyl) 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycer*o-α-D-*galacto*-2-nonulopyranosid]onate: (19)

Compound **18** (450 mg, 0.865 mmol), Cbz-amino propanol (271 mg, 1.297 mmol) and 3Å molecular sieves (450 mg) were suspended in dry MeCN (10 mL) and stirred under argon atmosphere with cooling, until temperature reached –40°C. NIS (231 mg, 12.0 mmol) and trifluoromethane sulfonic acid (52 mg, 0.346 mmol) were added successively. After stirring for 30 min, the reaction was warmed to –30°C and stirring was continued at this temperature for additional 14 h. The mixture was diluted with DCM and filtered through a pad of Celite. After subsequent washing with 20% aqueous Na$_2$S$_2$O$_3$ (20 mL) and sat. aqueous NaHCO$_3$ (20 mL), the organic layer was dried over Na$_2$SO$_4$, filtered and concentrated to give a syrup. Purification by column chromatography on silica gel (petrol ether/DCM/2-propanol 8:4:1) yielded **19** (624 mg) as a crude mixture of α:β isomer (1:0.4) and some residual impurities.

Methyl [(3-NHCbz-propyl) 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzamido-3,5,9-trideoxy-D-*glycer*o-α-D-*galacto*-2-nonulopyranosid] onate: (20)

According to the general method **I**, **19** (624 mg, 0.938 mmol) was reacted with benzoyl chloride (525 mg, 3.75 mmol) and triphenyl phosphine (540 mg, 2.06

mmol) in DCE (20 mL) for 14 h. After workup **20** was obtained as a crude solid (620 mg, 94%) with a mixture of α:β isomer (1:0.7).

(3-NHCbz-propyl) 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside: (21)

To a solution of **20** (624 mg) in methanol (25 mL) was added 10% aq. NaOH (2.4 mL). The mixture was stirred at r.t. for 3 h. The solution was neutralized with 10% aq. HCL (2.4 ml) and then concentrated. The residue was purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water) to give first **21** as α-isomer (85 mg, 17%) and in later fractions the β isomer.

^{1}H NMR (500MHz, D_2O): δ 1.67 (m, 2H, H-3a, H-2'), 2.00 (s, 3H, NHAc), 2.72 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 3.13 (m, 2H, H-3'), 3.44 (m, 2H, H-9a, H-1'a), 3.53 (dd, *J* = 1.1, 8.9 Hz, 1H, H-7), 3.64-3.76 (m, 4H, H-4, H-6, H-9a, H-1'b), 3.74 (m, 1H, H-5), 3.92 (m, 1H, H-8), 4.90 (s, 2H, H-1") 7.25, 7.31, 7.41, 7.52, 7.65 (m, 10H, Ar-H).

(3-Amino-propyl) 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside: (22)

The compound **21** (85 mg, 0.140 mmol) was dissolved in MeOH (5 ml) and stirred under argon atmosphere for 10 min. The flask was saturated with H_2 atmosphere and stirred for 2 hrs. According to TLC, hydrogenation was complete. The reaction mixture was filtered through a pad of celite and a microfilter to get the compound **22** (66 mg, 95 %) as a white solid.

^{1}H NMR (500MHz, D_2O): δ 1.66 (m, 1H, H-3a), 1.76 (m, 2H, H-2'), 1.97 (s, 3H, NHAc), 2.71 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 2.82 (m, 2H, H-3'), 3.51 (m, 3H, H-

7, H-9a, H-1'a), 3.65 (m, 1H, H-4), 3.79 (m, 4H, H-5, H-6, H-9b, H-1'b), 4.03 (m, 1H, H-8), 7.49, 7.58, 7.74 (m, 5H, Ar-H).

[3-Amino-(4-carboxy-TEMPO)-propyl] 5-acetamido-4,7,8-tri-O-acetyl-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside: (12a*)

Dhbt-OH (33.7 mg, 0.207) and DIC (26.08 mg, 0.207mmol) were added to the solution of the 1-Oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine (41.4 mg, 0.207mmol) in dry THF (2 mL). The mixture was stirred at 0°C for 2 h. The solvent was evaporated and quickly dried in vacuum. Dry DMF (1 mL) was added to the solid followed by N,N-Diisopropylethylamine (DIPEA, 26.7 mg, 0.207 mmol) and the **22** (65 mg, 0.138 mmol). After stirring the reaction mixture for 3 hours at room temperature, the TLC (DCM:MeOH 10:5) showed completion of reaction. The solvents were removed under high vaccum and the residue was purified by reversed phase chromatography on LiCroPrep® RP-8 (5% gradient of MeOH:H_2O) coulmn. After P2 size exclusion chromatography and lyophilization from water, **23** (52.0 mg, 57%) was obtained as a white solid

For analysis purposes phenyl hydrazine (0.005 mL) was added to the sample (2 mg) in CD_3OD. The solvent was evaporated and the sample D_2O exchanged. Impurity peaks were estimated at less than 5%.

^{1}H NMR (500MHz, D_2O): δ 1.14-1.27 (m 12H, 4XCH_3), 1.64 (m, 2H, H-3a, H-2''a), 1.72 (m, 2H, H-2', H-2''b), 1.98 (s, 3H, NHAc), 2.69 (m, 1H, H-1''), 2.72 (dd, J = 4.8, 12.5 Hz, 1H, H-3e), 3.23 (m, 2H, H-3'), 3.49-3.57 (m, 3H, H-7, H-9a, H-1'a), 3.67 (m, 1H, H-4), 3.75 (m, 3H, H-6, H-9a, H-1'b), 3.82 (m, 1H, H-5), 4.02 (m, 1H, H-8), 7.51, 7.60, 7.75 (m, 5H, Ar-H).

Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate: (24)

To a stirred solution of **2** (800 mg, 1.500 mmol) I) was added and AcCl (1.8 ml) in DCM (5 ml) in a "bombenrohr", 32% HCl (213 at -20°C. After stirring for 16 h at r.t., the mixture was diluted with cold DCM and subsequently washed with H_2O, 5% $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated to give **24** (700 mg, 91 %) as a fluffy solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.95 (s, 3H, NHAc), 2.08, 2.09, 2.11, 2.16 (4s, 12H, OAc), 2.32 (t, J = 13.9 Hz, 1H, H-3a), 2.82 (dd, J = 4.8, 13.9 Hz, 1H, H-3e), 3.91 (s, 3H, CO_2CH_3), 4.10 (dd, J = 5.7, 12.5 Hz, 1H, H-9a), 4.24 (q, J = 10.5, 1H, H-5), 4.38 (dd, J = 2.4, 10.8 Hz, 1H, H-6), 4.45 (dd, J = 2.7, 12.6 Hz, 1H, H-9b), 5.21 (m, 1H, H-8), 5.36 (d, J = 10.2 Hz, 1H, NH), 5.45 (m, 1H, H-4), 5.51 (dd, J = 2.4, 7.1 Hz, 1H, H-7).

Methyl (2-propynyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α- and β-D-*galacto*-2-nonulopyranosid) onate: (25a)

Compound **24** (1.40 g, 2.75 mmol) was dissolved in dry CH_3CN (30 ml) containing propargyl alcohol (308 mg, 5.50 mmol) and molecular sieves 3Å (4.0 g). The mixture was stirred at r.t. for one hour with light exclusion. Then AgOTf (1.41 g, 5.50 mmol) was added in one portion and stirring continued at r.t. for 16 h. The mixture was filtered through a pad of celite and the celite was washed thoroughly with DCM. The filtrate was subsequently washed with 10% $NaHCO_3$, sat. $Na_2S_2O_3$ and water. The organic layer was dried with Na_2SO_4 and the DCM evaporated. The residue was purified by silica gel column chromatography (PE:DCM:iPrOH 8:4:1) system. The compound **25a** (1.150 g, 79%) was obtained as anomeric mixture (α β 1:1), together with approximately 20% of glycal. MS-ESI: 552.27(M^++Na)

Methyl (2-propynyl 5-acetamido-3,5-dideoxy-D-*glycero*-α- and β-D-*galacto*-2-nonulopyranosid) onate: (26a)

A solution of **25a** (1.15 g, 2.20 mmol) in dry methanol (30 mL) was treated with 1 M methanolic NaOMe (5 mL) at r.t. for 2 h. The reaction mixture was neutralized with Amberlyst 15 (H$^+$) ion-exchange resin and filtered through a pad of celite. The celite was thoroughly washed with methanol (3 × 10 mL), and the combined filtrates were evaporated to dryness. The residue was purified by silica gel column chromatography (DCM:MeOH 7:3) to afford anomeric mixture of **26a**, α-isomer (263 mg, 33%) and β-isomer as a mixture with glycal (500 mg, 62%). MS-ESI: 360.14, 384.10 (M$^+$+Na)

α-isomer: ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.71 (t, *J* = 12.6 Hz, 1H, H-3a), 1.97 (s, 3H, NHAc), 2.67 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.83 (X of ABX, *J* = 2.4 Hz, 1H, C≡CH), 3.47 (dd, *J* = 1.3, 9.0 Hz, 1H, H-7,), 3.56 (dd, *J* = 1.3, 10.5 Hz, 1H, H-6), 3.60-3.65 (m, 2H, H-4, H-9a), 3.73-3.83 (m, 3H, H-5, H-8, H-9b), 3.81 (s, 3H, CO$_2$CH$_3$), 4.32 (AB of ABX, *J* = 2.4, 15.9 Hz, 2H, H-1'), ^{13}C NMR (125 MHz, D$_2$O): δ 23.2 (NHCOCH$_3$), 41.9 (C-3), 53.3 (C-1'), 53.9 (CO$_2$CH$_3$), 54.1 (C-5), 65.1 (C-9), 68.9 (C-4), 70.5 (C-7), 72.6 (C-8), 75.3 (C≡CH), 75.3 (C-6), 80.7 (C≡CH), 99.9 (C-2), 170.9, 175.6 (2CO).

Methyl [2-propynyl 5-acetamido-3,5-dideoxy-9-*O*-(4-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid] onate: (27a)

To the solution of a-isomer of **26a** (263 mg, 0.728 mmol) in pyridine (15 ml) was added *p*-TsCl (138 mg, 0.728 mmol) at 0°C. After 2 h, *p*-TsCl (69 mg, 0.364 mmol) was added and stirring continued for 16 h at 5°C. The reaction mixture was warmed to r.t., then diluted with methanol (20 mL) and stirring continued for 30 min. After removal of solvents, the remaining syrup was purified by

chromatography on silica gel (DCM/MeOH 19:1) to yield **27a** as a solid (223 mg, 60%).

MS-ESI: 538.20 (M$^+$+Na) ^{1}H NMR (500MHz, CD$_3$OD): δ 1.69 (t, *J* = 12.8 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.46 (s, 3H, Ph-C$\underline{H}_3$), 2.67 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.84 (X of ABX, *J* = 2.5 Hz, 1H, C≡C$\underline{H}$) 3.44 (dd, *J* = 1.4, 8.8 Hz, 1H, H-7), 3.55 (dd, *J* = 1.4, 10.5 Hz, 1H, H-6), 3.63 (m, 1H, H-4), 3.71 (m, 1H, H-5), 3.81 (s, 3H, CO$_2$CH$_3$), 3.97 (m 1H, H-8), 4.08 (m, 1H, H-9a), 4.23-4.32 (m, 3H, H-9b, H-1'), 7.45 (AA' of AA'BB', 2H, *J* = 8.0 Hz, Ar-H), 7.80 (BB' of AA'BB', 2H, *J* = 8.3 Hz, Ar-H), ^{13}C NMR (125 MHz, D$_2$O): δ 22.0 (Ph-C$\underline{H}_3$), 23.1 (NHCOC$\underline{H}_3$), 41.9 (C-3), 53.4 (C-1'), 53.7 (CO$_2$CH$_3$), 54.0 (C-5), 68.7 (C-4), 70.1 (C-8), 70.4 (C-7), 74.1 (C-9), 75.0 (C-6), 76.1 (C≡$\underline{C}$H), 80.8 ($\underline{C}$≡CH), 100.0 (C-2), 129.5, 131.4, 134.7, 146.8 (6C, C$_6$H$_5$), 170.5, 175.5 (2CO).

Methyl (2-propynyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo pyranosid) onate: (28a)

A mixture of **27a** (223 mg, 0.433 mmol), crown ether 18-C-6 (44.8 mg, 0.170 mmol) and NaN$_3$ (140 mg, 2.15 mmol) was stirred in DMF (4 mL) at 60°C for 24 h. TLC analysis (DCM:MeOH 9:1) showed completion of the reaction. The mixture was filtered through a pad of celite and the filtrate was evaporated to dryness. Purification by column chromatography on silica gel (DCM:MeOH 7:3) yielded **28a** as a white solid (110 mg, 66%).

MS-ESI: 387.11, 385.09. ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.76 (t, *J* = 12.6 Hz, 1H, H-3a), 2.03 (s, 3H, NHAc), 2.72 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.87 (X of ABX, *J* = 2.5 Hz, 1H, C≡C$\underline{H}$), 3.41 (dd, *J* = 6.2, 12.8 Hz, 1H, H-9a), 3.49 (dd, *J* = 1.4, 9.0 Hz, 1H, H-7), 3.57 (dd, *J* = 2.6, 12.8 Hz, 1H, H-9b), 3.64 (dd, *J* = 1.5, 10.5 Hz, 1H, H-6), 3.68 (m, 1H, H-4), 3.79 (m, 1H, H-5), 3.87 (s, 3H, CO$_2$CH$_3$), 4.01 (m, 1H, H-8), 4.38 (AB of ABX, *J* = 2.5 Hz, 15.8 Hz, 2H, H-1'). ^{13}C NMR (125 MHz, D$_2$O): δ 23.1 (NHCOC$\underline{H}_3$), 41.9 (C-3), 53.5 (C-1'), 53.9 (CO$_2$C$\underline{H}_3$), 54.1 (C-

5), 55.9 (C-9), 68.8 (C-4), 71.3 (C-7), 71.6 (C-8), 75.1 (C-6), 76.1 (C≡$\underline{C}$H), 80.8 ($\underline{C}$≡CH), 100.0 (C-2), 170.7, 175.6 (2CO).

Methyl (2-propynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (29a)

Compound **28a** (110 mg, 0.284 mmol) was dissolved in dry pyridine (1.17 ml, 14.8 mmol) and stirred at 0°C for 15 min under argon before DMAP (4.90 mg, 0.040 mmol) and Ac$_2$O (1.28 ml, 12.6 mmol) were added simultaneously at 0°C. After stirring at r.t. for 14 h, the solvent was evaporated. The residual solid was purified by column chromatography on silica gel (DCM/MeOH 9:1) to get **29a** as white foam (90 mg, 62%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.84 (s, 3H, NHAc), 1.95 (t, *J* = 12.5 Hz, 1H, H-3a), 2.00, 2.12, 2.14 (3s, 9H, 3OAc), 2.43 (X of ABX, *J* = 2.4 Hz, 1H, C≡C$\underline{H}$), 2.60 (dd, *J* = 4.7, 12.9 Hz, 1H, H-3e), 3.25 (dd, *J* = 2.9, 5.2 Hz, 1H, H-9a), 3.55 (dd, *J* = 2.5, 14.7 Hz, 1H, H-9b), 3.78 (s, 3H, CO$_2$CH$_3$), 4.06 (m, 2H, H-5, H-6), 4.12, 4.35 (AB of ABX, *J* = 2.4, 15.7 Hz, 2H, H-1'), 4.83 (m, 1H, H-4), 5.28 (m, 2H, H-7, H-8), 5.48 (d, *J* = 9.3 Hz, 1H, NH), ^{13}C NMR (125 MHz, D$_2$O): δ 21.2, 21.2, 21.4 (3OCO$\underline{C}$H$_3$), 23.5 (NHCO$\underline{C}$H$_3$), 38.1 (C-3), 49.5 (C-5), 51.2 (C-9), 53.1 (CO$_2$$\underline{C}H_3$), 53.3 (C-1'), 68.2 (C-8), 69.1 (C-4), 70.2 (C-7), 73.3 (C-6), 75.0 (C≡$\underline{C}$H), 79.2 ($\underline{C}$≡CH), 98.5 (C-2), 168.1, 170.6, 170.6 170.7, 171.3 (5CO).
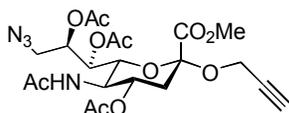
Methyl (2-propynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzamido-3,5,9-trideoxy)-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (30a)
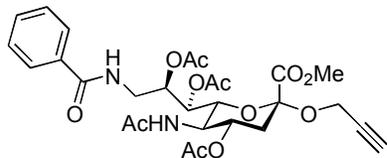
Compound **29a** (90.0 mg, 0.175 mmol) was reacted with benzoyl chloride (95.0 mg, 0.678 mmol) and triphenyl phosphine (97.0 mg, 0.370 mmol) in DCE (4 mL) at r.t. for 16 h. The reaction mixture was diluted with DCM and subsequently

washed with 10% NaHCO$_3$ and water. The organic layer was dried with Na2SO4. The solvent was evaporated and the residue was purified by column chromatography on silica gel (DCM:MeOH 97:3) to get **30a** as a white solid (54 mg, 52 %).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.88 (s, 3H, NHAc), 1.98 (t, *J* = 12.6 Hz, 1H, H-3a), 2.03, 2.09, 2.24 (3s, 9H, 3OAc), 2.43 (X of ABX, *J* = 2.4 Hz, 1H, C≡CH), 2.64 (dd, *J* = 4.6, 12.7 Hz, 1H, H-3e), 2.97 (m, 1H, H-9a), 3.73 (s, 3H, CO$_2$CH$_3$), 3.99 (dd, *J* = 2.1, 10.8 Hz, 1H, H-6), 4.16 (m, 2H, H-5, H-1'a), 4.32 (m, 1H, H-9b), 4.39 (dd, *J* = 2.5, 15.6 Hz, 1H, H-1'a), 4.85 (m, 1H, H-4), 5.16 (dd, *J* = 2.0, 9.8 Hz, 1H, H-7), 5.27 (m, 1H, H-8), 5.60 (d, *J* = 10.1 Hz, 1H, NH-5), 7.11 (m, 1H, NH-9), 7.44, 7.58, 7.81 (m, 5H, C$_6$H$_5$), ^{13}C NMR (125 MHz, D$_2$O): δ 21.2, 21.4, 21.5 (3OCOCH$_3$), 23.4 (NHCOCH$_3$), 38.1 (C-3), 38.8 (C-9), 49.7 (C-5), 53.2 (2C, CO$_2$CH$_3$, C-1'), 68.2 (C-7), 68.7 (C-8), 69.3 (C-4), 72.6 (C-6), 74.9 (C≡CH), 79.2 (C≡CH), 98.4 (C-2), 127.3, 128.8, 130.4, 133.8 (6C, C$_6$H$_5$), 167.9, 167.9, 170.8 171.1, 171.6, 172.7 (6CO).

Sodium (2-propynyl 5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (31a)
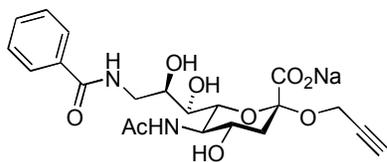
To a solution of compound **30a** (54 mg, 0.091 mmol) in methanol (2 mL) was added 10% aq. NaOH (0.2 mL). The mixture was stirred at r.t. for 3 h. After neutralization with 10 % HCl (0.2 ml), the solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na$^+$ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **31a** (41 mg, 95%) as a white solid after a final lyophilization from water.

$[\alpha]_D^{25}$ = - 0.5 (*c* = 0.10, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.67 (t, *J* = 12.2 Hz, 1H, H-3a), 1.98 (s, 3H, NHAc), 2.73 (dd, *J* = 4.7, 12.5 Hz, 1H, H-3e), 3.54 (m, 2H, H 7, H-9a), 3.67 (m, 1H, H-4), 3.75 (m, 2H, H-6, H-9b), 3.83 (m, 1H, H-5), 4.06 (m

1H, H-8), 4.35, 4.21 (A, B, of AB, J = 15.6 Hz, 2H, of H-1'), 7.50, 7.58, 7.74 (m, 5H, C_6H_5). ^{13}C NMR (125 MHz, D_2O): δ 22.3 (NHCOC$\underline{H}_3$), 40.6 (C-3), 43.0 (C-9), 52.1 (C-5), 52.9 (C-1'), 68.4 (C-4), 70.1 (C-7), 70.4 (2C, C-8, C≡$\underline{C}$H), 73.1 (C-6), 79.8 ($\underline{C}$≡CH), 101.0 (C-2), 127.4, 129.1, 132.4, 133.9 (C_6H_5), 171.6, 173.1, 175.3 (3CO).

Methyl (3-butynyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α- and β-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate: (25b)
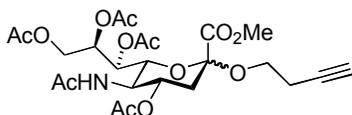
Compound **24** (910 mg, 1.78 mmol) was dissolved in dry CH_3CN (15 ml) containing 3-butynyl alcohol (270 mg, 3.85 mmol) and molecular sieves 3Å (1.80 g). The mixture was stirred at r.t. for 1 hr with light exclusion. Then AgOTf (914 mg, 3.55 mmol) was added in one portion and stirring continued at r.t. for 16 h. The suspension was filtered through a pad of celite and the celite was washed thoroughly with DCM. The filtrate was subsequently washed with 10% $NaHCO_3$, sat. $Na_2S_2O_3$ and water. The organic layer was dried with Na_2SO_4 and the solvent was evaporated to get compound **25b** (547 mg, 56%) as an anomeric mixture (α:β 1:1) along with glycal impurities. The residue was purified by silica gel column chromatography (PE:DCM:iPrOH 8:4:1) system. The early fractions were collected to get a mixture of α:β isomers (422 mg), which were taken as such for further reaction.

MS-ESI: 566.14 (M$^+$+Na) **β-isomer:** ^1H NMR (500 MHz, CDCl$_3$): δ 1.87 (m, 4H, H-3a, NHAc), 2.01, 2.03, 2.07 (3s, 9H, 3OAc), 2.13 (m, 1H, C≡C$\underline{H}$), 2.14 (s, 3H, OAc), 2.44-2.51(m, 3H, H-3e, H-2'), 3.49 (m, 1H, H-1'a), 3.61 (m, 1H, H-1'b), 3.80 (s, 3H, CO$_2$C$\underline{H}_3$), 4.11 (m, 2H, H-5, H-9a), 4.20 (dd, J = 1.9, 10.6 Hz, H-6), 4.82 (dd, J = 2.2, 12.5 Hz, 1H, H-9b), 5.21-5.32 (m, 2H, H-4, H-8), 5.40 (m, 2H, H-7, NH). ^{13}C NMR (125 MHz, D_2O): δ 20.0 (C-2'), 21.1, 21.2, 21.3, 21.5 (4OCOC$\underline{H}_3$), 23.5 (NHCOC$\underline{H}_3$), 37.7 (C-3), 49.6 (C-5), 53.1 (CO$_2$CH$_3$), 62.5 (C-1'), 62.7 (C-9), 68.6 (C-7), 69.2 (C-4), 70.6 (C≡$\underline{C}$H), 72.0 (C-6), 72.5 (C-8), 81.8 ($\underline{C}$≡CH), 98.7 (C-2), 167.0, 170.6, 170.7, 171.0, 171.1, 171.4 (6CO). **α-isomer:**

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.79 (s, 3H, NHAc), 1.87 (m, 2H, H-3a, C≡C<u>H</u>), 1.94, 1.96, 2.05, 2.06 (4s, 12H, 4OAc), 2.38 (m, 2H, H-2'), 2.52 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.32 (m, 1H, H-1'a), 3.72 (m, 3H, CO$_2$C<u>H</u>$_3$), 3.78 (m, 1H, H-1'b), 4.05 (m, 3H, H-5, H-6, H-9a), 4.21 (dd, *J* = 2.6, 12.4 Hz, 1H, H-9b), 4.78 (m, 1H, H-4), 5.07 (d, *J* = 9.4 Hz, 1H, NH), 5.23 (dd, *J* = 1.8, 8.4 Hz, 1H, H-7), 5.31 (m, 1H, H-8). ^{13}C NMR (125 MHz, D$_2$O): δ 20.0 (C-2'), 21.1, 21.2, 21.3, 21.4 (4OCOC<u>H</u>$_3$) 23.5 (NHCOC<u>H</u>$_3$), 38.4 (C-3), 49.6 (C-5) 53.1 (CO$_2$C<u>H</u>$_3$), 62.7 (C-9), 63.7 (C-1'), 67.7 (C-7), 69.1 (C-8), 69.4 (C≡<u>C</u>H), 69.7 (C-4), 72.9 (C-6), 81.1 (<u>C</u>≡CH), 99.1 (C-2), 168.6, 170.5, 170.6, 170.7, 171.0, 171.3 (6CO).

Methyl (3-butynyl 5-acetamido -3,5-dideoxy-D-*glycero*-α- and β-D-*galacto*-2-nonulopyranosid) onate: (26b)
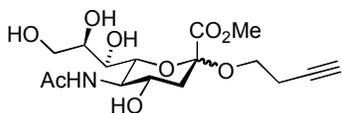
A solution of **25b** (422 mg, 0.777 mmol) in dry methanol (10 mL) was treated with 1 M methanolic NaOMe (1.5 mL) at r.t. for 2 h. The reaction mixture was neutralized with Amberlyst 15 (H^{+}) ion-exchange resin and filtered through a pad of celite. The celite was washed thoroughly with methanol (3 × 5 mL), and the combined filtrates were evaporated and the residue purified by silica gel column chromatography (DCM:MeOH 7:3) system. The initial fractions collected gave α-isomer **26b** (165 mg, 56%) and the remaining fractions gave β ˜ (145 mg, 49%) with some impurities.

MS-ESI: 374.08, 376.10. **α-isomer:** ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.73 (t, *J* = 12.4 Hz, 1H, H-3a), 2.00 (s, 3H, NHAc), 2.26 (m, 1H, C≡C<u>H</u>), 2.41 (m, 2H, H-2'), 2.69 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.51 (m, 3H, H-7, H-9a, H-1'a), 3.57 (d, *J* = 10.5 Hz, 1H, H-6), 3.65 (m, 2H, H-4, H-9b), 3.76 (m, 1H, H-5), 3.87 (m, 6H, H-8, H-9b, H-1'b, CO$_2$CH$_3$), ^{13}C NMR (125 MHz, D$_2$O): δ 21.03 (C-2'), 23.0 (NHCOC<u>H</u>$_3$), 42.0 (C-3), 53.8 (CO$_2$CH$_3$), 54.2 (C-5), 64.1 (C-1'), 65.1 (C-9), 68.9 (C-4), 70.5 (C-7), 71.0 (C≡<u>C</u>H), 72.8 (C-8), 75.3 (C-6), 82.5 (<u>C</u>≡CH), 100.6 (C-2), 171.2, 175.6 (2CO). **β-isomer:** ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.80 (t, *J* = 12.8 Hz, 1H, H-3a), 2.01 (s, 3H, NHAc), 2.26 (m, 1H, C≡C<u>H</u>), 2.43 (m, 2H, H-2'), 2.39

(dd, *J* = 4.9, 13.0 Hz, 1H, H-3e), 3.37 (m, 1H, H-1'a), 3.51 (d, *J* = 9.3 Hz, 1H, H-7), 3.65 (dd, *J* = 5.1, 11.2 Hz, 1H, H-9a), 3.80-3.99 (m, 8H, H-5, H-6, H-8, H-9b, H-1'b, CO$_2$CH$_3$), 4.06 (m, H-4). ^{13}C NMR (125 MHz, CD$_3$OD): δ 19.5 (C-2'), 23.1 (NHCOCH$_3$), 40.8 (C-3), 52.3 (CO$_2$CH$_3$), 53.0 (C-5), 62.1 (C-1'), 64.4 (C-9), 66.8 (C-4), 69.2 (C-7), 69.8 (C≡CH), 70.6 (C-8), 71.7 (C-6), 81.0 (C≡CH), 99.2 (C-2), 169.8, 174.1 (2CO).

Methyl [(3-butynyl 5-acetamido-3,5-dideoxy-9-*O*-(4-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid] onate: (27b)
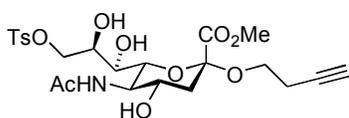
To the solution of a-isomer of **26b** (165 mg, 0.440 mmol) in pyridine (10 ml) was added *p*-TsCl (83.6 mg, 0. 440 mmol) at 0°C. After 2 h, *p*-TsCl (41.8 mg, 0.220 mmol) was added and stirring continued for 16 h at 5°C. The reaction mixture was warmed to r.t., diluted with methanol (20 mL) and stirring continued for 30 min. After removal of the solvents the remaining syrup was purified by chromatography on silica gel (DCM/MeOH 19:1) to yield **27b** as a white solid (167 mg, 72%).

MS-ESI: 552.17(M$^+$+Na), 528.31. ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.69 (t, *J* = 12.4 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.26 (m, 1H, C≡CH,) 2.38 (m, 2H, H-2'), 2.46 (s, 3H, Ph-CH$_3$), 2.65 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.44 (d, *J* = 1.3, 8.8 Hz, 1H, H-7), 3.48 (m, 1H, H-1'a), 3.55 (dd, *J* = 1.3, 10.4 Hz, 1H, H-6), 3.63 (m, 1H, H-4), 3.71 (m, H-5), 3.80 (m, 4H, H-1'b, CO$_2$CH$_3$), 3.97 (m 1H, H-8), 4.08 (m, 1H, H-9a), 4.31 (dd, 1H, *J* = 2.1, 10.1 Hz, H-9b), 7.45, 7.80 (AA'BB', *J* = 8.3 Hz, 4H, C$_6$H$_5$), ^{13}C NMR (125 MHz, CD$_3$OD): δ 21.0 (C-2'), 22.0 (Ph-CH$_3$), 23.1 (NHCOCH$_3$), 42.0 (C-3), 53.6 (CO$_2$CH$_3$), 54.1 (C-5), 64.1 (C-1'), 68.8 (C-4), 70.2 (C-8), 70.4 (C-7), 71.0 (C≡CH), 74.0 (C-9), 74.9 (C-6), 82.0 (C≡CH), 100.7 (C-2), 129.5, 131.4, 134.7, 146.8 (6C, C$_6$H$_4$), 170.9, 175.6 (2CO).

Methyl (3-butynyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (28b)
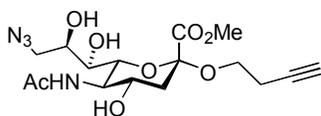
A mixture of **27b** (167 mg, 0.315 mmol), crown ether 18-C-6 (31.6 mg, 0.120 mmol) and NaN$_3$ (100 mg, 1.55 mmol) was stirred in DMF (6 mL) at 60°C for 24 h. The solvent was evaporated and the residue purified by column chromatography on silica gel (DCM:acetone 7:3) to give **28b** as a white solid (90 mg, 71 %).

MS-ESI: 401.15, 399.19. ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.74 (t, *J* = 12.3 Hz, 1H, H-3a), 2.03 (s, 3H, NHAc), 2.28 (t, *J* = 2.6 Hz, 1H, C≡CH̲), 2.43 (m, 2H, H-2'), 2.70 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.40 (dd, *J* = 6.2, 12.8 Hz, 1H, H-9a), 3.43-3.56 (m, 3H, H-7, H-9b, H-1'a), 3.64 (m, 2H, H-4, H-6), 3.77 (m, 1H, H-5), 3.87 (s, 4H, H-1'b, CO$_2$CH$_3$), 3.99 (m 1H, H-8). ^{13}C NMR (125 MHz, D$_2$O): δ 21.0 (C-2'), 23.1 (NHCOC̲H$_3$), 42.0 (C-3), 53.9 (CO$_2$CH$_3$), 54.2 (C-5), 55.8 (C-9), 64.1 (C-1'), 68.9 (C-4), 71.1 (C-7), 71.2 (C≡C̲H), 71.8 (C-8), 75.0 (C-6), 82.0 (C̲≡CH), 100.6 (C-2), 171.5, 175.6 (2CO).

Methyl (3-butynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (29b)
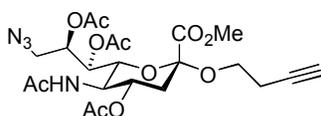
The compound **28b** (90 mg, 0.22 mmol) was dissolved in dry pyridine (903 mg, 11.4 mmol) and stirred at 0°C for 15 min under argon before DMAP (3.66 mg, 0.03 mmol) and Ac$_2$O (1.00 g, 9.90 mmol) were added simultaneously at 0°C and stirring continued at r.t. for 14 h. The reaction mixture was diluted with DCM and subsequently washed with 10% HCl, 10% NaHCO$_3$ and water. The organic layer was dried with Na$_2$SO$_4$. After evaporating the solvent, the residue was purified on silica gel (DCM/MeOH 20:1) to get **29b** as a white solid (90mg, 76%).

MS-ESI: 571.18 (M$^+$+Na). ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.88 (s, 3H, NHAc), 1.98 (m, 2H, H-3a, C≡CH̲), 2.02, 2.16, 2.17 (3s, 9H, 3OAc), 2.45 (m, 2H, H-2'), 2.60 (dd, *J* = 4.6, 12.9 Hz, 1H, H-3e), 3.29 (dd, *J* = 3.6, 5.7 Hz, 1H, H-9a), 3.42 (m, 1H, H-1'b), 3.59 (dd, *J* = 2.7, 13.3 Hz, 1H, H-9b), 3.81 (s, 3H, CO$_2$CH$_3$), 3.85 (m,

142

1H, H-1'b), 4.09 (m, 2H, H-5, H-6), 4.85 (m, 1H, H-4), 5.30 (m, 3H, H-7, H-8, NH), ^{13}C NMR (125 MHz, D$_2$O): δ 20.3 (C-2'), 21.2, 21.2, 21.4 (3C, OCO$\underline{C}$H$_3$), 23.5 (NHCO$\underline{C}$H$_3$), 38.2 (C-3), 49.7 (C-5), 51.2 (C-9), 53.2 (CO$_2\underline{C}$H$_3$), 63.5 (C-1'), 68.4 (C-7), 69.3 (C-4), 69.8 (C≡$\underline{C}$H), 70.4 (C-8), 73.3 (C-6), 81.0 ($\underline{C}$≡CH), 99.2 (C-2), 168.6, 170.5, 170.6 170.7, 171.4 (5CO).

Methyl (3-butynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzamido-3,5,9-trideoxy)-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (30b)
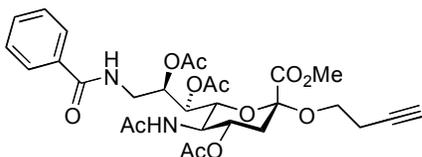
Compound **29b** (90.0 mg, 0.171 mmol) was reacted with benzoyl chloride (95.0 mg, 0.678 mmol) and triphenyl phosphine (97.0 mg, 0.370 mmol) in DCE (3 mL). After stirring at r.t. for 16 h, diluted with DCM and washed with 10% NaHCO$_3$. The organic layer was dried with Na$_2$SO$_4$. After evaporating the solvent, the residue was purified by column chromatography on silica gel (DCM/MeOH 9.7:0.3) to get **30b** (72 mg, 69%) as a white solid.

MS-ESI: 627.26 (M$^+$+Na), 603.27. ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.87 (s, 3H, NHAc), 1.97 (m, 2H, H-3a, C≡C$\underline{H}$), 2.02, 2.10, 2.24 (3s, 9H, 3OAc), 2.44 (m, 2H, H-2'), 2.61 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 2.94 (m, 1H, H-9a), 3.40 (m, 1H, H-1'a), 3.75 (s, 3H, CO$_2$CH$_3$), 3.88 (m, 1H, H-1'b), 4.00 (dd, *J* = 2.0, 10.7 Hz, 1H, H-6), 4.17 (q, *J* = 10.7 Hz, 1H, H-5), 4.34 (m, 1H, H-9b), 4.82 (m, 1H, H-4), 5.14 (dd, *J* = 2.0, 9.7 Hz, 1H, H-7), 5.25 (m, 1H, H-8), 5.37 (d, *J* = 10.1 Hz, 1H, NH-5), 7.09 (m, 1H, NH-9), 7.40, 7.49, 7.81 (m, 5H, C$_6$H$_5$), ^{13}C NMR (125 MHz, D$_2$O): δ 20.4 (C-4'), 21.2, 21.4, 21.5 (OCO$\underline{C}$H$_3$), 23.5 (NHCO$\underline{C}$H$_3$), 38.2 (C-3), 38.7 (C-9), 49.8 (C-5), 53.1 (COO$\underline{C}$H$_3$), 63.4 (C-1'), 68.3 (C-7), 68.8 (C-8), 69.4 (C-4), 69.8 (C≡$\underline{C}$H), 72.5 (C-6), 81.2 ($\underline{C}$≡CH), 99.0 (C-2), 127.3, 128.9, 131.8, 134.6 (6C, C$_6$H$_5$), 167.6, 168.4, 170.6 170.7, 171.5, 172.7 (6CO).

Sodium (3-butynyl 5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (31b)
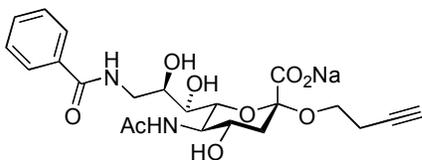
To a solution of compound **30b** (72 mg, 0.119 mmol) in methanol (5 mL) was added 10% aq. NaOH (0.5 mL). The mixture was stirred at r.t. for 3 h. After the evaporating the solvents, the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na^+ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **31b** (41 mg, 72%) as a colorless solid after a final lyophilization from water.

$[\alpha]_D^{25}$ = - 1.7 (*c* = 0.10, H_2O); MS-ESI: 627.26 (M^++Na), 603.27. ^{1}H NMR (500 MHz, D_2O): δ 1.69 (t, *J* = 12.2 Hz, 1H, H-3a), 2.00 (s, 3H, NHAc), 2.34 (m, 3H, C≡CH), 2.36 (m, 2H, H-2'), 2.74 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 3.55 (m, 3H, H-7, H-9a, H-1'a), 3.69 (m, 1H, H-4), 3.75-3.86 (m, 4H, H-5, H-6, H-9b, H-1'b), 4.05 (m 1H, H-8), 7.50, 7.60, 7.65 (m, 5H, C_6H_5). ^{13}C NMR (125 MHz, D_2O): δ 19.6 (C 2'), 23.3 (NHCO$\underline{C}$H₃), 40.6 (C-3), 43.0 (C-9), 52.2 (C-5), 63.2 (C-1'), 68.5 (C-4), 70.2 (C-7), 70.5 (C-8), 70.7 (C≡$\underline{C}$H), 72.9 (C-6), 82.6 ($\underline{C}$≡CH), 101.1 (C-2), 127.4, 129.1, 132.4, 133.9 (6C, C_6H_5), 171.5, 173.7, 175.7 (3CO).

Methyl (4-pentynyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-ᴅ-*glycero*-α- and β-ᴅ-*galacto*-2-nonulopyranosid) onate: (25c)
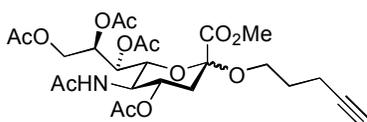
Compound **24** (1.40 g, 2.75 mmol) was dissolved in dry CH_3CN (30 ml) containing 3-pentynyl alcohol (462 mg, 5.50 mmol) and molecular sieves 3Å (1.80 g). This was stirred at r.t. for 1 hr with light exclusion. Then AgOTf (1.41 g, 5.50 mmol) was added in one portion and stirring continued at r.t. for 16 h. The mixture was diluted with DCM (30 ml) and the residue filtered through a pad of celite. The celite was washed thoroughly with DCM and the filtrate was subsequently washed with 10% $NaHCO_3$, sat. $Na_2S_2O_3$ and water. The organic layer was dried with Na_2SO_4 and the DCM. After evaporating the solvent, the residue was purified by silica gel column chromatography (PE:DCM:iPrOH 8:4:1)

to get a compound **25c** (1.11 g, 56%) as anomeric mixture (α β 1:1) with approximately 25% of glycal.

Methyl (3-pentynyl 5-acetamido-3,5-dideoxy-D-*glycero*-α- and β-D-*galacto*-2-nonulopyranosid) onate: (26c)
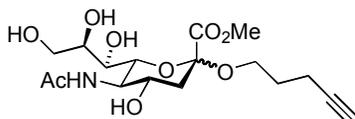
A solution of **25c** (775 mg, 1.39 mmol) in dry methanol (20 mL) was treated with 1 M methanolic NaOMe (3 mL) at r.t. for 2 h. The reaction mixture was neutralized with Amberlyst 15 (H$^+$) ion-exchange resin and filtered through a pad of celite. The celite was thoroughly washed with methanol (3 × 10 mL) and the filtrate was evaporated. The residue was purified by silica gel column chromatography (DCM:MeOH 7:3) to yield anomeric mixture of **26c** with α-isomer (242 mg, 44%) and β-isomer (120 mg, 22%).

α-isomer: ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.72 (m, 3H, H-3a, H-2'), 2.00 (s, 3H, NHAc), 2.23 (m, 3H, H-3', C≡C$\underline{H}$) 2.68 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.48 (m, 1H, H-1'a), 3.51 (dd, *J* = 1.6, 8.8 Hz, 1H, H-7), 3.58 (d, *J* = 1.6, 10.4 Hz, H-6), 3.63 (m, 2H, H-4, H-9a), 3.76 (m, 1H, H-5), 3.84 (m, 3H, CO$_2$CH$_3$), 3.85 (m, 3H, H-8, H-9b, H-1'b). ^{13}C NMR (125 MHz, CD$_3$OD): δ 16.0 (C-3'), 23.0 (NHCO$\underline{C}$H$_3$), 30.2 (C-2'), 42.1 (C-3), 53.8 (CO$_2$CH$_3$), 54.2 (C-5), 64.0 (C-1'), 65.0 (C-9), 68.9 (C-4), 70.2 (C≡$\underline{C}$H), 70.6 (C-7), 72.9 (C-8), 75.3 (C-6), 84.7 ($\underline{C}$≡CH), 100.6 (C-2), 171.5, 175.6 (2XCO). **β-Isomer:** ^{1}H NMR (500MHz, CD$_3$OD): δ 1.63 (t, *J* = 12.7 Hz, 1H, H-3a), 1.69-1.80 (m, 2H, H-2'), 2.01 (s, 3H, NHAc), 2.22 (m, 1H, C≡C$\underline{H}$), 2.24-2.35 (m, 2H, H-3'), 2.39 (dd, *J* = 4.8, 12.8 Hz, 1H, H-3e), 3.31 (m, 1H, H-1'a), 3.49 (d, *J* = 9.1 Hz, 1H, H-7), 3.69 (m, 1H, H-9a), 3.69-3.89 (m, 8H, H-5, H-6, H-8, H-9b, H-1'b, CO$_2$C$\underline{H}$$_3$), 3.96 (m, 1H, H-4). ^{13}C NMR (125 MHz, CD$_3$OD): δ 16.3 (C-3'), 23.2 (NHCO$\underline{C}$H$_3$), 30.4 (C-2'), 42.1 (C-3), 53.9 (CO$_2$CH$_3$), 54.1 (C 5), 63.5 (C-1'), 65.6 (C-9), 68.1 (C-4), 70.1 (C≡$\underline{C}$H), 70.5 (C-7), 71.8 (C-8), 72.7 (C-6), 85.1 ($\underline{C}$≡CH), 100.4 (C-2), 171.4, 175.2 (2CO).

Methyl [4-pentynyl 5-acetamido-3,5-dideoxy-9-*O*-(4-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate: (27c).
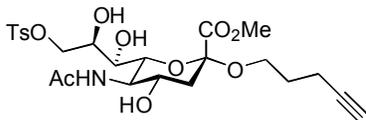
To a solution of **26c** (225 mg, 0.578 mmol) in pyridine (15 ml) was added *p*-TsCl (108 mg, 0.578 mmol) at 0°C. After 2 h, *p*-TsCl (53.0 mg, 0.289 mmol) was added and stirring continued for 16 h at 5°C. The reaction mixture was warmed to r.t., then diluted with methanol (20 mL) and stirring continued for 30 min. After removal of solvents the remaining syrup was purified by chromatography on silica gel (DCM:MeOH 19:1) to yield **27c** (240 mg, 76%) as a white solid.

MS-ESI: 566.34 (M$^+$+Na), 542.35. ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.70 (m, 3H, H-3a, H-2'), 2.02 (s, 3H, NHAc), 2.24 (m, 3H, H-3', C≡C$\underline{H}$), 2.47 (s, 3H, Ph-CH$_3$), 2.68 (dd, *J* = 4.7, 12.7, Hz, 1H, H-3e), 3.45 (m, 1H, H-1'a), 3.48 (dd, *J* = 1.4, 8.7 Hz, 1H, H-7), 3.59 (dd, *J* = 1.2, 10.4 Hz, 1H, H-6), 3.65 (m, 1H, H-4), 3.74 (m, 1H, H-5), 3.81 (m, 1H, H-1'b), 3.83 (s, 3H, CO$_2$CH$_3$), 4.04 (m, 1H, H-8), 4.10 (dd, *J* = 6.3, 10.0 Hz, 1H, H-9a), 4.35 (dd, *J* = 2.0, 10.0 Hz, 1H, H-9b), 7.46, 7.81 (AA', BB' of AA'BB', 4H, *J* = 8.4 Hz, C$_6$H$_4$). ^{13}C NMR (125 MHz, CD$_3$OD): δ 16.0 (C-3'), 22.1 (Ph-$\underline{C}$H$_3$), 23.2 (NHCO$\underline{C}$H$_3$), 30.21 (C-2'), 42.1 (C-3), 53.9 (CO$_2$CH$_3$), 54.2 (C-5), 64.1 (C-1'), 68.7 (C-4), 70.4 (C-7), 70.5 (2C, C-8, C≡$\underline{C}$H), 74.0 (C-9), 75.0 (C-6), 84.9 ($\underline{C}$≡CH), 100.7 (C-2), 129.5, 131.5, 134.5, 146.9 (6C, C$_6$H$_4$), 171.3, 175.7 (2CO).

Methyl (4-pentynyl 5-acetamido–9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate: (28c).
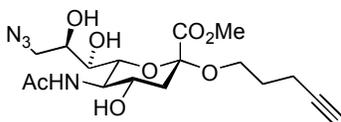
A mixture of **27c** (235 mg, 0.432 mmol), crown ether 18-C-6 (45.0 mg, 0.170 mmol) and NaN$_3$ (140 mg, 2.15 mmol) was stirred in DMF (7 mL) at 60°C for 24 h. The solvent was evaporated and the residual solid purified by column chromatography on silica gel (DCM:acetone 7:3) to give **28c** (150 mg, 83 %) as a white solid.

MS-ESI: 413.22, 415.23. ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.73 (m, 3H, H-3a, H-2'), 2.01 (s, 3H, NHAc), 2.24 (m, 3H, H-3', C≡C$\underline{H}$), 2.68 (dd, J = 4.6, 12.8, Hz, 1H, H-3e), 3.39 (dd, J = 6.4, 12.8 Hz, 1H, H-9a), 3.48 (m, 2H, H-7, H-1'a), 3.55 (dd, J = 2.6, 12.8 Hz, 1H, H-9b), 3.63 (m, 2H, H-4, H-6), 3.75 (m, 1H, H-5), 3.85 (s, 3H, CO$_2$CH$_3$), 3.86 (m, 1H, H-1'b), 4.00 (m, 1H, H-8), ^{13}C NMR (125 MHz, CD$_3$OD): δ 16.0 (C-3'), 23.2 (NHCO$\underline{C}$H$_3$), 30.2 (C-2'), 42.1 (C-3), 53.9 (CO$_2$CH$_3$), 54.3 (C-5), 55.7 (C-9), 64.0 (C-1'), 68.9 (C-4), 70.4 (C≡$\underline{C}$H), 71.3 (C-7), 71.9 (C-8), 75.0 (C-6), 84.9 ($\underline{C}$≡CH), 100.7 (C-2), 171.4, 175.7 (2CO).

Methyl (4-pentynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate: (29c).
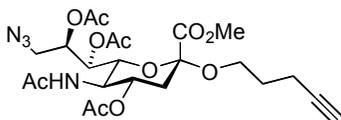
Compound **28c** (150 mg, 0.362 mmol) was dissolved in dry pyridine (1.50 g, 19.0 mmol) and stirred at 0°C for 15 min under argon before DMAP (7.32 mg, 0.060 mmol) and Ac$_2$O (1.65 g, 16.2 mmol) were added simultaneously at 0°C. After stirring at r.t. for 14 h, the reaction mixture was diluted with DCM. The reaction mixture was the subsequently washed with 10% HCl, 10% NaHCO$_3$ and water. The organic layer was dried with Na$_2$SO$_4$. After evaporating the solvent the residue was purified by column chromatography on silica gel (DCM:MeOH 20:1) to give **29c** (180 mg, 92%) as a white solid.

MS-ESI: 563.26 (M$^+$+Na). ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.67 (m, 3H, H-2'), 1.79 (s, 3H, NHAc), 1.81-1.89 (m, 2H, H-3a, C≡C$\underline{H}$), 1.94, 2.07, 2.08 (3s, 9H, 3OAc), 2.18 (m, 2H, H-3') 2.50 (dd, J = 4.7, 12.9, Hz, 1H, H-3e), 3.22 (dd, J = 6.4, 12.8 Hz, 1H, H-9a), 3.28 (m, 1H, H-1'a), 3.55 (dd, J = 2.9, 13.5 Hz, 1H, H-9b), 3.69 (m, 1H, H-1'b), 3.71 (s, 3H, CO$_2$CH$_3$), 4.03 (m, 2H, H-5, H-6), 4.75 (m, 1H, H-4), 5.19 (m, 1H, H-8), 5.25 (dd, J = 1.6, 6.6 Hz, 1H, H-7), 5.82 (d, J = 9.5 Hz, 1H, NH). ^{13}C NMR (125 MHz, CDCl$_3$): δ 15.2 (C-3'), 21.1, 21.3, 21.6 (3OCO$\underline{C}$H$_3$), 23.4 (NHCO$\underline{C}$H$_3$), 28.8 (C-2'), 38.1 (C-3), 49.4 (C-5) 51.0 (C-9), 53.1 (CO$_2$CH$_3$), 63.5 (C-1'), 68.4 (C-7), 69.0 (C-4), 69.5 (C≡$\underline{C}$H), 71.1 (C-8), 73.3 (C-6), 84.0 ($\underline{C}$≡CH), 99.2 (C-2), 168.7, 170.5, 170.7, 170.7, 171.3 (5CO).

Methyl (4-pentynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate: (30c).
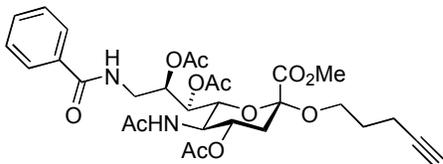
Compound **29c** (180 mg, 0.333 mmol) was reacted with benzoyl chloride (184 mg, 1.32 mmol) and triphenyl phosphine (189 mg, 0.721 mmol) in DCE (2 mL). After stirring at r.t. for 16 h, the solvent was evaporated. Purification by column chromatography on silica gel (DCM:MeOH 100:1 to 20:1) yielded **30c** as a white solid (146 mg, 70%).

MS-ESI: 641.32 (M^++Na), 617.37. ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.71 (m, 2H, H 2'), 1.83 (s, 3H, NHAc), 1.89 (m, 2H, H-3a, C≡C$\underline{H}$), 1.98, 2.05, 2.20 (3s, 9H, 3OAc), 2.22 (m, 2H, H-3'), 2.55 (dd, J = 4.6, 12.7 Hz, 1H, H-3e), 2.94 (ddd, J = 3.7, 7.6, 11.2 Hz, 1H, H-9a), 3.30 (m, 1H, H-1'a), 3.69 (s, 3H, CO$_2$C$\underline{H}_3$), 3.80 (m, 1H, H-1'b), 4.02 (dd, J = 2.0, 10.7 Hz, 1H, H-6), 4.14 (q, J = 10.7 Hz, 1H, H-5), 4.28 (ddd, J = 3.1, 6.7, 8.5 Hz, 1H, H-9b), 4.78 (m, 1H, H-4), 5.12 (dd, J = 2.0, 9.7 Hz, 1H, H-7), 5.23 (m, 1H, H-8), 5.67 (d, J = 10.0 Hz, 1H, NH-5), 7.11 (m, 1H, NH-9), 7.37, 7.44, 7.77 (m, 5H, C$_6$H$_5$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 15.3 (C-3'), 21.2, 21.4, 21.5 (3OCO$\underline{C}$H$_3$), 23.4 (NHCO$\underline{C}$H$_3$), 28.9 (C-2'), 38.3 (C-3), 38.8 (C-9), 49.7 (C-5), 53.0 (CO$_2$CH$_3$), 63.5 (C-1'), 68.3 (C-7), 68.9 (2C, C-8, C≡$\underline{C}$H), 69.5 (C-4), 72.4 (C-6), 84.1 ($\underline{C}$≡CH), 99.0 (C-2), 127.3, 128.7, 128.9, 130.3, 131.8, 134.5 (6C, C$_6$H$_5$), 167.7, 168.6, 170.6, 170.9, 171.4, 172.6 (6CO).

Sodium (4-pentynyl 5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate: (31c).
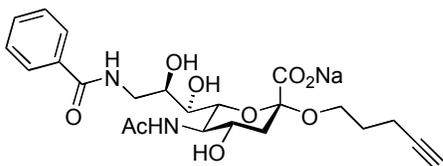
To a solution of compound **30c** (146 mg, 0.236 mmol) in methanol (10 mL) was added 10% aq. NaOH (0.6 mL). The mixture was stirred at r.t. for 3 h. The

solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na$^+$ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford **31c** (116 mg, 98%) as a white solid after a final lyophilization from water.

$[\alpha]_D^{25}$ = - 1.2 (*c* = 0.10, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.65-1.73 (m, 3H, H-3a, H-2'), 1.99 (s, 3H, NHAc), 2.19-2.26 (m, 2H, H-3'), 2.73 (dd, *J* = 4.7, 12.4 Hz, 1H, H-3e), 3.45-3.52 (m, 2H, H-9a, H-1'a), 3.55 (d, *J* = 1.4, 8.9 Hz, 1H, H-7), 3.67 (m, 1H, H-4), 3.75 (dd, *J* = 1.3, 10.5 Hz, 1H, H-6), 3.78-3.84 (m, 3H, H-5, H-9b, H 1'b), 4.05 (m 1H, H-8), 7.45, 7.55, 7.69 (m, 5H, C$_6$H$_5$). ^{13}C NMR (125 MHz, D$_2$O): δ 14.7 (C-3'), 22.4 (NHCO$\underline{C}$H$_3$), 28.3 (C-2'), 40.8 (C-3), 43.0 (C-9), 52.3 (C-5), 63.7 (C-1'), 68.6 (C-4), 70.3 (C-7), 70.8 (C-8), 72.9 (C-6), 85.0 ($\underline{C}$≡CH), 101.1 (C-2), 127.4, 129.1, 132.5, 133.8 (6C, C$_6$H$_5$), 171.2, 173.9, 175.4 (3CO).

Methyl (5-hexynyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate: (25d)
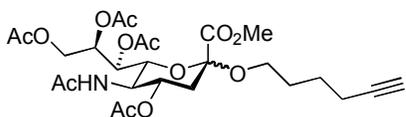
Compound **24** (970 mg, 1.90 mmol) was dissolved in dry CH$_3$CN (25 ml) containing 3-hexynyl alcohol (372 mg, 3.80 mmol) and molecular sieves 3Å (2.00 g). This was stirred at r.t. for 1 hr with light exclusion. Then AgOTf (976 mg, 3.80 mmol) was added in one portion and stirring continued at r.t. for 16 h. Diluted with DCM (30 ml). The mixture was filtered through a pad of celite and the celite was washed thoroughly with DCM. The filtrate was subsequently washed with 10% NaHCO$_3$, sat. Na$_2$S$_2$O$_3$ and water. The organic layer was dried with Na$_2$SO$_4$ and the DCM was evaporated. The residue was purified with silica gel column chromatography (PE:DCM:iPrOH 8:4:1) to give compound **25d** (678 mg, 62%) as anomeric mixture (α β 1:1) with approximately 20% of   .
MS-ESI: 594.26 (M$^+$+Na).

α-isomer: ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.55-1.65 (m, 4H, H-2', H-3'), 1.86 (s, 3H, NHAc), 1.92 (m, 2H, H-3a, C≡$\underline{C}$H), 2.00, 2.01, 2.02, 2.03 (4s, 12H, 4OAc), 2.20 (m, 2H, H-4'), 2.55 (dd, *J* = 3.3, 12.8 Hz, 1H, H-3e), 3.23 (m, 1H, H-1'a),

3.76 (m, 4H, H-1'b, CO$_2$CH$_3$), 4.06 (m, 3H, H-5, H-6, H-9a), 4.29 (d, J = 12.4 Hz, 1H, H-9b), 4.82 (m, 1H, H-4), 5.31 (m, 2H, H-7, NH-5), 5.36 (m, 1H, H-8). ^{13}C NMR (125 MHz, CDCl$_3$): δ 18.3 (C-4'), 21.1, 21.2, 21.3, 21.5 (4OCOCH$_3$), 23.5 (NHCOCH$_3$), 25.3 (C-3'), 28.9 (C-2'), 38.4 (C-3), 49.8 (C-5), 53.0 (CO$_2$CH$_3$), 62.7 (C-9), 64.7 (C-1'), 67.7 (C-7), 68.7 (C-8), 69.0 (C≡CH), 69.5 (C-4), 72.8 (C-6), 84.7 (C≡CH), 99.0 (C-2), 168.9, 170.4, 170.5, 170.6, 171.0, 171.4 (6CO). **β-isomer:** ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.59 (m, 2H, H-3'), 1.70 (m, 2H, H-2'), 1.85 (m, 1H, H-3a), 1.86 (s, 3H, NHAc), 1.91 (m, 1H, C≡CH) 2.00, 2.01, 2.05, 2.12 (4s, 12H, 4OAc), 2.22 (m, 2H, H-4'), 2.43 (dd, J = 4.9, 12.9 Hz, 1H, H-3e), 3.33 (m, 1H. H-1'a), 3.49 (m, 1H, H-1'b), 3.73 (s, 3H, CO$_2$CH$_3$), 3.87 (dd, J = 2.0, 10.5 Hz, 1H, H-6), 4.11 (m, 2H, H-5, H-9a), 4.79 (dd, J = 2.1, 12.4 Hz, H-9b), 5.16 (m, 1H, H-8), 5.24 (m, 1H, H-4), 5.38 (m, 1H, H-7), 5.44 (d, J = 10.2 Hz, 1H, NH), ^{13}C NMR (125 MHz, CDCl$_3$): δ 18.5 (C-4'), 21.1, 21.2, 21.3, 21.4 (4OCOCH$_3$), 23.5 (NHCOCH$_3$), 25.3 (C-3'), 28.8 (C-2'), 37.8 (C-3), 49.7 (C-5) 53.0 (CO$_2$CH$_3$), 62.7 (C-9), 63.9 (C-1'), 68.8 (C-7), 69.1 (C-4), 69.3 (C≡CH), 72.1 (C-6), 72.5 (C-8), 84.4 (C≡CH), 98.9 (C-2), 167.9, 170.5, 170.6, 170.9, 171.0, 171.4 (6CO).

Methyl (5-hexynyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid) onate: (26d).
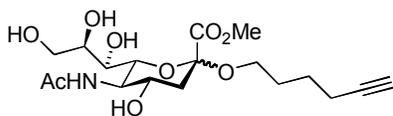
A solution of **25d** (374 mg, 0.654 mmol) in dry methanol (8.0 mL) was treated with 1 M methanolic NaOMe (0.8 mL) at r.t. for 2 h. The reaction mixture was neutralized with Amberlyst 15 (H$^+$) ion-exchange resin and filtered through a pad of celite. The celite was thoroughly washed with methanol (3 × 5 mL), and the combined filtrates were evaporated. The residue was purified by silica gel column chromatography (DCM:MeOH 7:3) to yield anomeric mixture of **26d** with α-isomer (200 mg, 76%) and β̃ (50 mg, 19%).

MS-ESI: 426.18 (M$^+$+Na), 402.22. **α-isomer:** ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.58 (m, 2H, H-3'), 1.67 (m, 2H, H-2'), 1.77 (t, J = 12.4 Hz, 1H, H-3a), 2.04 (s, 3H,

NHAc), 2.20 (m, 3H, H-4', C≡C$\underline{H}$), 2.70 (dd, J = 4.6, 12.8 Hz, 1H, H-3e), 3.42 (m, 1H, H-1'a), 3.55 (d, J = 9.0 Hz, 1H, H-7), 3.60 (d, J = 1.4, 10.4 Hz, 1H, H-6), 3.66 (m, 2H, H-4, H-9a), 3.77–3.90 (m, 4H, H-5, H-8, H-9b, H-1'b), 3.88 (m, 3H, CO$_2$CH$_3$). ^{13}C NMR (125 MHz, CD$_3$OD): δ 19.0 (C-4'), 23.0 (NHCOC$\underline{H}_3$), 26.6 (C-3'), 30.0 (C-2'), 42.1 (C-3), 53.8 (CO$_2$C$\underline{H}_3$), 54.2 (C-5), 65.0 (C-1'), 65.1 (C-9), 68.9 (C-4), 70.1 (C-7), 70.6 (C≡C$\underline{H}$), 72.9 (C-8), 75.3 (C-6), 85.2 ($\underline{C}$≡CH), 100.6 (C-2), 171.6, 175.6 (2CO). **β-isomer:** ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.60-1.72 (m, 5H, H-3a, H-2', H-3'), 2.04 (s, 3H, NHAc), 2.22 (m, 3H, H-4', C≡C$\underline{H}$), 2.41 (dd, J = 4.9, 12.9 Hz, 1H, H-3e), 3.24 (m, 1H, H-1'a), 3.53 (d, J = 8.9 Hz, 1H, H-7), 3.69 (m, 1H, H-9a), 3.84 (m, 8H, H-5, H-6, H-8, H-9b, H-1'b, CO$_2$CH$_3$), 4.04 (m, H-4). ^{13}C NMR (125 MHz, CD$_3$OD): δ 19.3 (C-4'), 23.1 (NHCOC$\underline{H}_3$), 26.6 (C-3'), 30.1 (C-2'), 42.2 (C-3), 53.6 (CO$_2$C$\underline{H}_3$), 54.2 (C-5), 64.4 (C-1'), 65.6 (C-9), 68.1 (C-4), 70.0 (C-7), 70.5 (C≡C$\underline{H}$), 71.8 (C-8), 72.7 (C-6), 85.3 ($\underline{C}$≡CH), 100.4 (C-2), 171.6, 175.2 (2CO).

Methyl (5-hexynyl 5-acetamido-3,5-dideoxy-9-*O*-(4-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (27d)
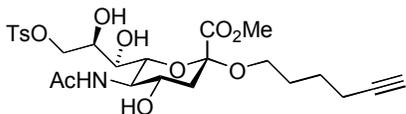
To a solution of **26d** (200 mg, 0.496 mmol) in pyridine (10 ml) was added *p*-TsCl (93.0 mg, 0. 496 mmol) at 0°C. After 2 h, *p*-TsCl (46.0 mg, 0.248 mmol) was added and stirring continued for 16 h at 5°C. The reaction mixture was warmed to r.t., then diluted with methanol (10 mL) and stirring continued for 30 min. After removal of solvents the remaining syrup was purified by chromatography on silica gel (DCM:MeOH 19:1) to yield **27d** (182 mg, 66%) as a white solid.

MS-ESI: 580.18 (M$^+$+Na), 556.24. ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.52 (m, 2H, H-3'), 1.60 (m, 2H, H-2'), 1.68 (t, J = 12.4 Hz, 1H, H-3a), 1.98 (s, 3H, NHAc), 2.20 (m, 3H, H-4', C≡C$\underline{H}$), 2.45 (s, 3H, Ph-C$\underline{H}_3$), 2.63 (dd, J = 4.6, 12.8 Hz, 1H, H-3e), 3.33 (m, 1H, H-1'b), 3.45 (d, J = 8.5 Hz, 1H, H-7), 3.54 (d, J = 10.4 Hz, 1H, H-6), 3.60 (m, 1H, H-4), 3.72 (m, 2H, H-5, H-1b'), 3.80 (s, 3H, CO$_2$CH$_3$), 4.06 (m 1H, H-8), 4.09 (dd, J = 6.2, 10.0 Hz, 1H, H-9a), 4.31 (dd, J = 2.9, 10.0 Hz, 1H, H-9b),

7.47, 7.82 (AA', BB' of AA'BB', 4H, J = 8.2 Hz, Ar-H), ^{13}C NMR (125 MHz, CD$_3$OD): δ 19.0 (C-4'), 22.0 (Ph-$\underline{C}$H$_3$), 23.1 (NHCO$\underline{C}$H$_3$), 26.5 (C-3'), 30.0 (C-2'), 42.0 (C-3), 53.7 (CO$_2$CH$_3$), 54.2 (C-5), 65.1 (C-1'), 68.8 (C-4), 70.1 (C-7), 70.4 (C≡$\underline{C}$H), 70.5 (C-8), 74.0 (C-9), 74.9 (C-6), 85.3 ($\underline{C}$≡CH), 100.7 (C-2), 129.5, 131.5, 134.6, 146.8 (6C, C$_6$H$_4$), 171.3, 175.7 (2CO).

Methyl (5-hexynyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (28d)
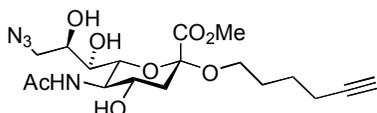
A mixture of **27d** (180 mg, 0.323 mmol), crown ether 18-C-6 (32.0 mg, 0.121 mmol) and NaN$_3$ (104 mg, 1.60 mmol) was stirred in DMF (8 mL) at 60°C for 24 h. The solvent was evaporated and the residual solid was purified by column chromatography on silica gel (DCM:acetone 7:3) to give **28d** (112 mg, 81 %) as a white solid.

MS-ESI: 427.22, 451.19 (M$^+$+Na). ^{1}H NMR (500 MHz, CD$_3$OD): δ 1.58 (m, 2H, H-3'), 1.66 (m, 2H, H-2'), 1.76 (t, J = 12.4 Hz, 1H, H-3a), 2.04 (s, 3H, NHAc), 2.22 (m, 3H, H-4', C≡C$\underline{H}$), 2.70 (dd, J = 4.7, 12.8 Hz, 1H, H-3e), 3.41 (m, 2H, H-9a, H 1'a), 3.58 (dd, J = 2.6, 12.8 Hz, 1H, H-7), 3.61 (dd, J = 1.5, 10.4 Hz, 1H, H-9b), 3.66 (m, 1H, H-4, H-6), 3.81 (m, 2H, H-5, H-1'b), 3.87 (s, 3H, CO$_2$CH$_3$), 4.01 (m 1H, H-8), ^{13}C NMR (125 MHz, CD$_3$OD): δ 19.1 (C-4'), 23.1 (NHCO$\underline{C}$H$_3$), 26.5 (C 3'), 30.1 (C-2'), 42.1 (C-3), 53.8 (CO$_2$CH$_3$), 54.2 (C-5), 55.7 (C-9), 65.1 (C-1'), 68.9 (C-4), 70.2 (C≡$\underline{C}$H), 71.3 (C-7), 71.9 (C-8), 75.0 (C-6), 85.2 ($\underline{C}$≡CH), 100.6 (C-2), 171.5, 175.7 (2CO).

Methyl (5-hexynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (29d)
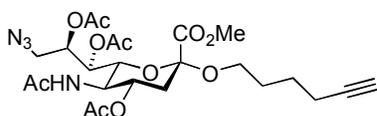
Compound **28d** (121 mg, 0.282 mmol) was dissolved in dry pyridine (1.10 g, 14.0 mmol) and stirred at 0°C for 15 min under argon before DMAP (6.00 mg, 0.049

mmol) and Ac$_2$O (1.22 g, 12.0 mmol) were added simultaneously at 0°C. After stirring at r.t. for 14 h, The reaction mixture was diluted with DCM and subsequently washed with 10% HCl, 10% NaHCO$_3$ and water. The organic layer was dried with Na$_2$SO$_4$. After evaporating the solvent, the residue was purified on silica gel (DCM/MeOH 20:1) to get **29d** (140 mg, 89%) as a white solid.

MS-ESI: 577.30 (M$^+$+Na). ^{1}H NMR (500MHz, CDCl$_3$): δ 1.53 (m, 2H, H-3'), 1.62 (m, 2H, H-2'), 1.82 (s, 3H, NHAc), 1.90 (m, 2H, H-3a, C≡CH̲), 1.98, 2.11, 2.12 (3s, 9H, 3OAc), 2.16 (m, 2H, H-4'), 2.53 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3e), 3.20-3.27 (m, 2H, H-9a, H-1'a), 3.56 (dd, *J* = 2.9, 13.5 Hz, 1H, H-9b), 3.70 (m, 1H, H-1'b), 3.75 (s, 3H, CO$_2$CH$_3$), 4.01-4.06 (m, 1H, H-5, H-6), 4.78 (m, 1H, H-4), 5.23 (m, 1H, H-8), 5.27 (m, 1H, H-7), 5.58 (m, 1H, NH-5). ^{13}C NMR (125 MHz, CDCl$_3$): δ 18.3 (C-4'), 21.1, 21.2, 21.3 (3OCOC̲H$_3$), 23.4 (NHCOC̲H$_3$), 25.3 (C-3'), 28.8 (C-2'), 38.3 (C-3), 49.5 (C-5), 51.1 (C-9), 53.0 (CO$_2$CH$_3$), 64.6 (C-1'), 68.4 (C-7), 68.8 (C≡C̲H), 69.4 (C-4), 70.9 (C-8), 73.2 (C-6), 84.6 (C̲≡CH), 99.1 (C-2), 168.8, 170.60, 170.64 170.7, 171.3 (5CO).

Methyl (5-hexynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-benzamido-3,5,9-trideoxy)-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (30d)
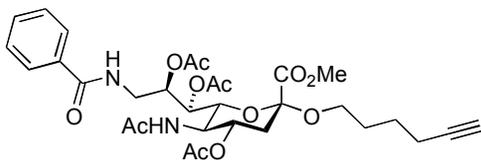
Compound **29d** (140 mg, 0.252 mmol) was reacted with benzoyl chloride (140 mg, 1.00 mmol) and triphenyl phosphine (145 mg, 0.554 mmol) in DCE (5 mL). After stirring at r.t. for 16 h, the reaction mixture was diluted with DCM and washed with 10% NaHCO$_3$ and water. The organic layer was dried with Na$_2$SO$_4$ and evaporated. Purification by column chromatography on silica gel (DCM:MeOH 100:1 to 20:1) yielded **30d** (100 mg, 64%) as a white solid.

MS-ESI: 631.33, 633.38. ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.52-1.63 (m, 4H, H-3', H-2'), 1.84 (s, 3H, NHAc), 1.91-1.94 (m, 2H, H-3a, C≡CH̲), 1.99, 2.07, 2.22 (3s, 9H, OAc), 2.13-2.20 (m, 2H, H-4'), 2.56 (dd, *J* = 4.6, 12.7 Hz, 1H, H-3e), 2.93 (m, 1H, H-9a), 3.22 (m, 1H, H-1'a), 3.72 (s, 3H, CO$_2$CH$_3$), 3.76 (m, 1H, H-1'b), 4.00 (dd, *J* = 2.0, 10.8 Hz, 1H, H-6), 4.14 (q, *J* = 10.8 Hz, 1H, H-5), 4.30 (m, 1H, H-9b), 4.77

(m, 1H, H-4), 5.12 (dd, J = 2.0, 9.6 Hz, 1H, H-7), 5.23 (m, 1H, H-8), 5.45 (m, 1H, NH-5), 7.09 (m, 1H, NH-9), 7.38-7.48, 7.86 (m, 5H, C_6H_5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.3 (C-4'), 21.2, 21.4, 21.5 (3OCO$\underline{C}H_3$), 23.4 (NHCO$\underline{C}H_3$), 25.3 (C-3'), 28.9 (C-2'), 38.4 (C-3), 38.7 (C-9), 49.8 (C-5), 53.0 (CO_2CH_3), 64.6 (C-1'), 68.3 (C-7), 68.8 (2C, C-8, C≡$\underline{C}$H), 69.5 (C-4), 72.4 (C-6), 84.7 ($\underline{C}$≡CH), 98.9 (C-2), 127.3, 128.5, 128.9, 131.8, 134.65 (6C, C_6H_5), 167.6, 168.7, 170.6 170.7, 171.4, 172.7 (6CO).

Sodium (5-hexynyl 5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosid) onate: (31d)
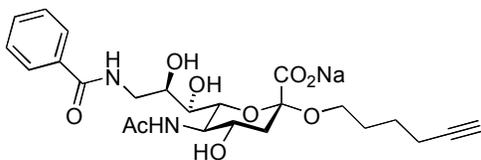
To a solution of compound **30d** (100 mg, 0.158 mmol) in methanol (4 mL) was added 10% aq. NaOH (0.4 mL). After stirring at r.t. for 3 h, the reaction mixture was neutralized with 10% HCl (0.4 mL). Then solution was concentrated and the residue purified by reversed phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 (Na$^+$ type) ion-exchange chromatography, and P2 size exclusion chromatography to afford 31d (76 mg, 95%) as a colorless solid after a final lyophilization from water.

$[\alpha]_D^{25}$ = - 0.7 (c = 0.10, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.43-1.52 (m, 2H, H-3'), 1.58-1.67 (m, 3H, H-3a, H-2'), 1.99 (s, 3H, NHAc), 2.14 (m, 2H, H-4'), 2.73 (dd, J = 4.6, 12.4 Hz, 1H, H-3e), 3.43 (m, 1H, H-1'a), 3.30 (dd, J = 4.6, 12.4 Hz, 1H, H-9a), 3.55 (dd, J = 1.7, 8.9 Hz, 1H, H-7), 3.67 (m, 1H, H-4), 3.72-3.84 (m, 4H, H-5, H-6, H-9b, H-1'b), 4.02 (m 1H, H-8), 7.47, 7.57, 7.72 (m, 5H, C_6H_5). ^{13}C NMR (125 MHz, D_2O): δ 17.5 (C-4'), 22.4 (NHCO$\underline{C}H_3$), 24.5 (C-3'), 28.5 (C-2'), 40.8 (C-3), 43.0 (C-9), 52.3 (C-5), 64.7 (C-1'), 68.6 (C-4), 70.3 (C-7), 70.8 (C-8), 72.9 (C-6), 85.7 ($\underline{C}$≡CH), 101.1 (C-2), 127.4, 129.1, 132.4, 133.9 (6C), 171.3, 173.9, 175.4 (3CO).

3-Carboxaldehyde-5-Nitro-1H-Indole: (33)
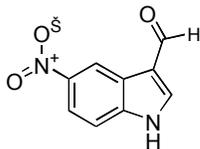
Dimethyl formamide (3.0 mL) was cooled to 0°C in an ice-salt bath and phosphorous oxychloride (0.8 mL) was added slowly over 30 min. The 5-nitro indole (1.40 gm, 8.64 mmol) in DMF (1.0 mL) was added slowly to the cooled solution over a period of 10 min and the resulting slurry heated at 35°C for 60 min until the clear yellow solution became yellowish paste. To this was added ice and 3.5 mL of 10% NaOH solution. This was heated to boil and late cooled in ice bath. The residual solid cake was filtered and vaccum dried to get **33** (1.52 gm, 92%).

^{1}H NMR (500 MHz, DMSO-d6): δ 7.72 (d, J = 9.0 Hz, 1H, Ar-H), 8.15 (m, 1H, Ar-H), 8.57 (s, 1H, Ar-H), 8.94 (s, 1H, Ar-H), 10.02 (s, 1H, Ar-H), 12.71 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d6): δ 113.6, 117.4, 119.1, 119.4, 123.9, 140.5, 1141.8, 143.2 (Ar-C), 185.9 (CO).

5-Nitro-3-(1H-Indole)Carbinol - (34)
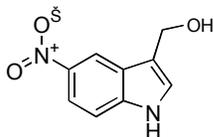
To a solution of compound **33** (1.52 gm, 8.00 mmol) in methanol (10 mL) was added NaBH$_4$ till the starting material is consumed as observed by TLC. This was added to ice cold water (50 mL). The residual yellow coloured solid was filtered and dried by vacccum to get compound **34** (1.42 mg, 92%).

^{1}H NMR (500 MHz, DMSO-d6): δ 4.70 (d, J = 4.8 Hz, 2H, CH$_2$), 5.04 (t, J = 5.0 Hz, 1H, OH), 7.52 (m, 1H, Ar-H), 8.00 (dd, J = 2.2, 9.0 Hz, 1H, Ar-H), 8.63 (d, J = 2.2 Hz, 1H, Ar-H), 11.64 (bs, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d6): δ 55.7 (CH$_2$), 112.1, 117.0, 117.5, 119.2, 126.7, 127.6, 128.0, 140.4 (Ar-C).

5-Nitro-3-(N-tertBOC-indole)carbinol: (35)
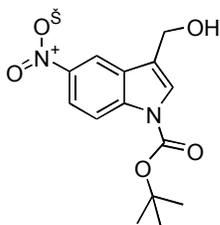
To a suspension of compound **34** (1.42 gm, 7.39 mmol) in dichoromethane (20 mL) was added *tert*-BOC anhydride (1.93 gm, 8.86 mmol) and DMAP (450 mg, 3.69 mmol). The reaction mixture was stirred for 60 min after which the TLC showed complete consumption of starting material. Solvents were evaporated and the residual solid was purified by silica gel column chromatography (EA:Pet-ether 1:4) to get **35** (1.33 gm, 61%).

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.68 (s, 9H, 3XCH$_3$), 4.91 (s, 2H, CH$_2$), 7.72 (s, 1H, Ar-H), 8.23 (m, 2H, Ar-H), 8.58 (d, *J* = 1.8 Hz, 1H, Ar-H). ^{13}C NMR (125 MHz, CDCl$_3$): δ 28.5 (3XC̲H$_3$), 57.1 (C̲H$_2$), 85.6 [C̲(CH$_3$)], 115.8, 116.3, 120.3, 121.4, 126.8, 129.5 (Ar-C).

3-bromomethyl-5-Nitro-*N*tBOC-indole: (37)
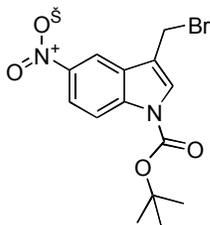
To a solution of compound **35** (660 mg, 2.26 mmol) in chloroform was added halo-enamine **36** under Argon atmosphere. This was stirred for 60 min after which the TLC showed complete consumption of **35**. Solvent was evaporated and the residual solid purified by silica gel column chromatography (EA:Pet-ether 1:9) to get compound **37** (475 mg, 60%) as a solid.

^{1}H NMR (500 MHz, CDCl$_3$): δ 1.68 (s, 9H, 3XCH$_3$), 4.67 (s, 2H, CH$_2$), 7.81 (s, 1H, Ar-H), 8.27 (m, 2H, Ar-H), 8.60 (d, *J* = 1.0 Hz, 1H, Ar-H). ^{13}C NMR (125 MHz, CDCl$_3$): δ 23.1 (C̲H$_2$), 28.5 (3XC̲H$_3$), 75.6 [C̲(CH$_3$)], 116.1, 116.2, 120.7, 128.0 (Ar-C).

3-(azidomethyl)-5-nitro-*N*tBOC-indole: (38)
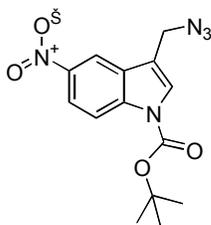
Compound **37** (475 mg, 1.34 mmol) was added to a solution of sodium azide (104 mg, 1.60 mmol) and 15-C-5-crown ether (116 mg, 0.53 mmol) in DMF (10 mL). The reaction mixture was stirred for 16 h after solvent was evaporated and the residual solid purified by silica gel chromatography (EA:Pet-ether 1:9) to get **38** (412 mg, 97%) as a solid.

^{1}H NMR (500 MHz, CDCl$_3$): δ 2.00 (s, 9H, 3XCH$_3$), 4.53 (s, 2H, CH$_2$), 7.75 (s, 1H, Ar-H), 8.27 (m, 2H, Ar-H), 8.53 (d, *J* = 0.8 Hz, 1H, Ar-H).

3-(azidomethyl)-5-nitro-1H-indole: (39)
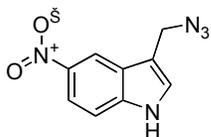
To a solution of compound **38** (470 mg, 1.48 mmol) in dichloromethane (20 mL) was added TFA (1 mL). This was stirred for 16 h at r.t. after which the solvents were evaporated. To this residual liquied was added ice-cold water. The solid was filtered and dried under high vaccum. The solid was further purified by silica gel column chromatography (EA:Pet-ether 9:1) to get **39** (190 mg, 70%).

^{1}H NMR (500 MHz, DMSO-d6): δ 4.50 (s, 2H, CH$_2$), 7.39 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.55 (d, *J* = 2.4 Hz, 1H, 1H, Ar-H), 7.84 (dd, *J* = 2.3, 9.0 Hz, 1H, Ar-H), 8.45 (d, *J* = 2.3 Hz, 1H, Ar-H), 11.69 (bs, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d6): δ 45.9 (CH$_2$), 112.4, 113.2, 116.6, 117.9, 126.7, 130.3, 140.3, 141.7 (Ar-C).

Ethyl 5-nitro-3-(1H-indole)propionate: (40)
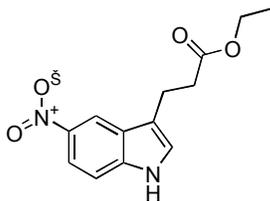
A solution of 5-nitroindole (200 g, 1.22 mmol), Meldrum's acid (210 mg, 1.46

mmol), 37% aqueous formaldehyde (43.8 mg, 1.46 mmol) and proline (14.9 mg, 0.06 mmol) in 0.8 mL of acetonitrile was stirred at room temperature for 18 h. The resulting thick yellow slurry was filtered and the filtercake was washed with acetonitrile, then acetone and finally with ether. This material was dried in vacuo to give [5-(5-nitroindol-3-yl)methyl]-2,2-dimethyl-1,3-dioxane-4,6-dione (355 mg, 81%) as a bright yellow solid.

To the solution of above crude compound (465 mg, 1.46 mmol) in a mixture of pyridine (34.6 mg, 0.438 mmol) and absolute ethanol (0.644 mg, 0.014) was added 4.65 mg of copper powder and the mixture was heated to reflux under Ar for 2 h. The cooled mixture was filtered and the filtrate was evaporated. The resulting residue was purified by silica gel chromatography (EA:Pet-ether 0.5:9.5) to give **40** (162 mg, 42%) as a solid.

^{1}H NMR (500 MHz, DMSO-d6): δ 1.14 (t, J = 7.2 Hz, 3H, CH$_3$), 2.68 (d, J = 7.4 Hz, 2H, H-1'), 3.03 (t, J = 7.4 Hz, 1H, H-2'), 4.04 (q, J = 7.2 Hz, 1H, CH$_2$), 7.41 (d, J = 2.0 Hz, 1H, Ar-H), 7.50 (d, J = 8.9 Hz, 1H, Ar-H), 7.98 (dd, J = 2.2, 8.9 Hz, 1H, Ar-H), 8.53 (d, J = 2.1 Hz, 1H, Ar-H), 11.60 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d6): δ 14.2 (C-CH$_3$), 20.1 (C-1'), 34.8 (C-2'), 60.1 (CH$_2$), 112.1, 116.0, 116.6, 116.8, 126.6, 126.8 (C-9), 139.6, 140.5 (Ar-C), 172.8 (CO).

5-Nitro-3-(3-hydroxypropyl)-1H-indole: (41)
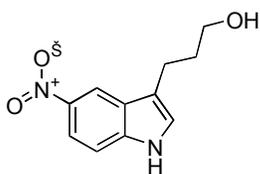
To a suspension of 95% LiAlH$_4$ (46.3 mg, 1.22 mmol) in 4 mL of dry THF was added a solution of ethyl 5-nitro-3-(1H-indole)propionate **40** (160 mg, 0.61 mmol) in 6 mL of dry THF, at 0 under Ar. After stirring for 20 min, the mixture was quenched by the cautious addition of 3 mL of H$_2$O. The resulting suspension was stirred for 10 min and then it was filtered and the filtercake was washed with additional THF. The filtrate was evaporated and the residue was taken up in ether, dried by Na$_2$SO$_4$ and evaporated. The resulting solid was triturated with hexane to give **41** (110 mg, 82%) as a yellow solid.

^{1}H NMR (500 MHz, MeOD): δ 1.92 (m, 2H, H-2'), 2.84 (t, J = 7.7 Hz, 2H, H-1'),

3.62 (t, *J* = 6.5 Hz, 2H, H-3'), 7.22 (s, 1H, Ar-H), 7.39 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.99 (dd, *J* = 2.2, 9.0 Hz, 1H, Ar-H), 8.52 (d, *J* = 2.1 Hz, 1H, Ar-H), ^{13}C NMR (125 MHz, MeOD): δ 22.4 (C-1'), 34.6 (C-2'), 62.8 (C-3'), 112.6, 117.1, 118.1, 119.5, 126.9, 128.5, 141.6, 142.5 (Ar-C).

3-(3-bromopropyl)- 5-Nitro-1H-indole: (42)
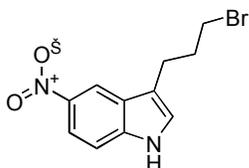
To a solution of compound **41** (110 mg, 0.50 mmol) in chloroform (4 mL) was added halo-enamine **36** (106.8 mg, 0.6 mmol) under Argon atmosphere. This was stirred for 60 min after which the TLC showed complete consumption of **41**. Solvent was evaporated and the residual solid purified by silica gel column chromatography (EA:Pet-ether 1:9) to get compound **42** (120 mg, 85%) as a solid.

^{1}H NMR (500 MHz, CDCl$_3$): δ 2.25 (m, 2H, H-2'), 2.97 (t, *J* = 7.4 Hz, 2H, H-1'), 3.44 (t, *J* = 6.4 Hz, 2H, H-3'), 7.21 (s, 1H, Ar-H), 7.41(d, *J* = 9.0 Hz, 1H, Ar-H), 8.11 (dd, *J* = 2.2, 9.0 Hz, 1H, Ar-H), 8.57 (m, 2H, Ar-H, NH). ^{13}C NMR (125 MHz, MeOD): δ 23.4 (C-1'), 33.0 (C-2'), 33.6 (C-3'), 111.6, 116.6, 117.7, 118.2, 125.2, 127.2, 139.7, 141.9 (Ar-C).

3-(3-Azidopropyl)- 5-Nitro-1H-indole: (43)
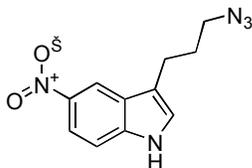
Compound **42** (120 mg, 0.425 mmol) was added to a solution of sodium azide (33.1 mg, 0.51 mmol) and 15-C-5-crown ether (37.4 mg, 0.17 mmol) in DMF (3.00 mL). The reaction mixture was stirred for 16 h after solvent was evaporated and the residual solid purified by silica gel chromatography (EA:Pet-ether 1:9) to get **43** (97.0 mg, 93%) as a solid.

^{1}H NMR (500 MHz, CDCl$_3$): δ 2.02 (m, 2H, H-2'), 2.90 (t, J = 7.6 Hz, 2H, H-1'), 3.36 (t, J = 6.7 Hz, 2H, H-3'), 7.18 (s, 1H, Ar-C), 7.40(d, J = 9.0 Hz, 1H, Ar-C), 8.11 (dd, J = 2.1, 9.0 Hz, 1H, Ar-C), 8.48 (s, 1H, NH), 8.57 (d, J = 1.8 Hz, 1H, Ar-C). ^{13}C NMR (125 MHz, MeOD): δ 22.2 (C-1'), 29.5 (C-2'), 51.1 (C-3'), 111.5, 116.6, 118.1, 118.2, 124.9, 127.2, 139.7, 141.9 (Ar-C).

1-(5-nitro-1H-indole-3-yl-methyl-)-4-{sodium [5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-O-yl]}methyl-[1,2,3]-triazole: (SA1NI1)
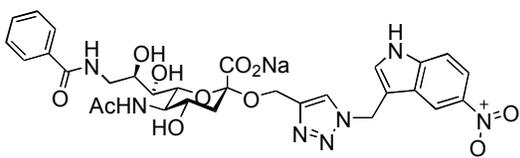
To a solution of **31a** (10.0 mg, 21.0 μmol) and **39** (4.55 mg, 21.0 μmol) in *tert*-BuOH:H$_2$O (1:1) (0.1 ml) was added Na-ascorbate (1.20 mg, 6.10 μmol, 6 μl of a freshly prepared 1M solution in H$_2$O), followed by CuSO$_4$·5H$_2$O (0.15 mg, 0.61 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which period at intervals four additional portions of Na-ascorbate (4.80 mg, 24.4 μmol in total) and three additional portions of CuSO$_4$.5H$_2$O (0.45 mg, 1.83 μmol) were added. After 36 h, according to TLC, the reaction was not finished. So a catalytic amount of CuCl$_2$ was added and stirring continued for 1 h after which TLC showed completion of the reaction. The solvents were removed under high vaccum and the residue was purified by reversed phase chromatography on LiCroPrep® RP-8 (5% gradient of MeOH:H$_2$O). After P2 size exclusion chromatography and lyophilization from water, **SA1NI1** (7.0 mg, 50%) was obtained as a white solid

$[\alpha]_D^{25}$ = - 0.4 (c = 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.66 (t, J = 12.1 Hz, 1H, H-3a), 2.00 (s, 3H, NHAc), 2.73 (dd, J = 4.6, 12.4 Hz, 1H, H-3e), 3.21 (m, 1H, H-9a), 3.47 (t, J = 7.4 Hz, 1H, H-7), 3.54 (m, 1H, H-9b), 3.64-3.76 (m, 3H, H-4, H-6, H-8), 3.81 (q, J = 10.0 Hz, 1H, H-5), 4.54, 4.84 (A, B of AB, J = 12.1 Hz, 2H, H-1'), 5.44 (d, J = 3.8 Hz, 2H, H-1''), 6.89 (d, J = 8.8 Hz, 1H, Ar-H), 7.14 (m, 2H, Ar-H), 7.25-7.37 (m, 7H, Ar-H), 7.80 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H). ^{13}C NMR (125

MHz, D$_2$O): δ 22.3 (NHCO$\underline{C}$H$_3$), 40.6 (C-3), 43.1 (C-9), 45.3 (C-1''), 52.1 (C-5), 57.9 (C-1'), 68.6 (C-4), 70.5 (C-7), 70.6 (C-8), 73.0 (C-6), 106.0 (C-2), 110.9, 111.7, 115.4, 117.4, 125.0, 127.0, 128.6, 129.2, 132.1, 133.0, 139.5, 140.4 (Ar-C), 170.4, 173.0, 175.3 (3CO).

1-(5-nitro-1H-indole-3-yl-methyl)-4-{sodium   [5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-O-yl]}-ethyl-[1,2,3] triazole: (SA2NI1)
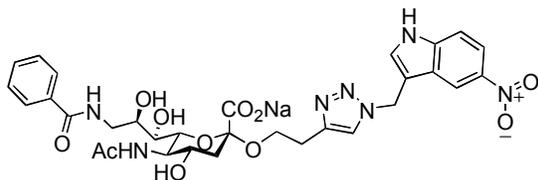
To a solution of **31b** (10.0 mg, 20.0 μmol) and **39** (4.34 mg, 20.0 μmol) in *tert*-BuOH:H$_2$O (1:1) (0.1 ml) was added Na-ascorbate (1.2 mg, 6.0 μmol, 6 μl of freshly prepared 1M solution in H$_2$O), followed by CuSO$_4$·5H$_2$O (0.15 mg, 0.60 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which period at intervals seven additional portions of Na-ascorbate (8.40 mg, 42.0 μmol in total) and three additional portions of CuSO$_4$.5H$_2$O (0.45 mg, 1.83 μmol in total) were added. After 36 h, according to TLC the reaction was not finished. So a catalytic amount of CuCl$_2$ was added and stirring continued for 1 h after which TLC showed completion of the reaction. The solvents were removed under high vaccum and the residue was purified by reversed phase chromatography on LiCroPrep® RP-8 (5% gradient if MeOH:H$_2$O). After P2 size exclusion chromatography and lyophilization from H$_2$O, **SA2NI1** (7.0 mg, 50%) was obtained as a white solid

$[\alpha]_D^{25}$ = - 0.4 (*c* = 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ1.50 (t, *J* = 12.1 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.64 (dd, *J* = 4.5, 12.4 Hz, 1H, H-3e), 2.85 (t, *J* = 6.3 Hz, 2H, H-2'), 3.32 (dd, *J* = 7.9, 13.9 Hz, 1H, H-9a), 3.48 (d, *J* = 8.8 Hz, 1H, H-7), 3.56 (dd, *J* = 2.7, 13.9 Hz, 1H, H-9b), 3.59-3.70 (m, 4H, H-4, H-5, H-6, H-1'a), 3.81 (m, 1H, H-8), 3.90 (m, 1H, H-1'b), 5.41 (d, *J* = 5.1 Hz, 2H, H-1''), 7.08 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.25 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.25 (m, 2H, Ar-H), 7.46 (d, *J* = 6.4 Hz, 2H, Ar-H), 7.51 (dd, *J* = 1.4, 9.0 Hz, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.91

(d, *J* = 1.2 Hz, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 22.3 (NHCO$\underline{C}$H$_3$), 26.0 (C-2'), 40.6 (C-3), 43.2 (C-9), 45.4 (C-1''), 52.1 (C-5), 63.6 (C-1'), 68.6 (C-4), 70.5 (2C, C-7, C-8), 72.9 (C-6), 101.0 (C-2), 111.1, 112.1, 115.7, 117.7, 124.0, 125.2, 127.1, 128.8, 129.2, 132.2, 133.4, 136.3, 139.8, 140.9 (Ar-C), 170.9, 173.8, 175.4 (3CO).

1-(5-nitro-1H-indole-3-methyl)-4-{sodium [5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-*O*-yl]}-propyl-[1,2,3]-triazole: (SA3NI1)
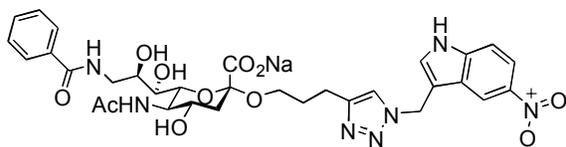
To solution of **31c** (9.0 mg, 18.0 μmol) and **39** (4.30 mg, 19.8 μmol) in *tert*-BuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.2 mg, 6.0 μmol, 6 μl of freshly prepared solution of 1M in H$_2$O), followed by CuSO$_4$.5H$_2$O (0.15 mg, 0.60 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which period at intervals was added three additional portions of Na-ascorbate (3.6 mg, 18.0 μmol in total) and one additional portion of CuSO$_4$.5H$_2$O (0.15 mg, 0.60 μmol) in H$_2$O. After 36 h, according to TLC the reaction was not complete. So a catalytic amount of CuCl$_2$ was added and stirring continued for 1 h after which TLC showed completion of the reaction. The solvents were removed under high vaccum and the residue was purified by reversed phase chromatography on LiCroPrep® RP-8 (5% gradient of MeOH:H$_2$O). After P2 size exclusion chromatography and lyophilization from H$_2$O, **SA3NI1** (6.0 mg, 46%) was obtained as a white solid.

$[\alpha]_D^{25}$ = -1.1 (*c* = 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.61 (t, *J* = 12.2 Hz, 1H, H-3a), 1.73-1.90 (m, 2H, H-2'), 2.00 (s, 3H, NHAc), 2.53-2.59 (m, 1H, H-3'a), 2.65-2.71 (m, 2H, H-3e, H-3'b), 3.30 (dd, *J* = 8.5, 13.9 Hz, 1H, H-9a), 3.40-3.52 (m, 3H, H-7, H-9b, H-1'a), 3.61-3.70 (m, 3H, H-4, H-6, H-1'b), 3.75-3.81 (m, 2H, H-5, H-8), 5.42 (d, *J* = 3.5 Hz, 2H, H-1''), 7.13 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.23 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.34-7.37 (m, 2H, Ar-H), 7.46-7.48 (m, 2H, Ar-H), 7.59 (dd, *J*

= 2.1, 9.0 Hz, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 7.93 (d, J = 2.1 Hz, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 21.3 (C-3'), 22.3 (NHCO$\underline{C}$H$_3$), 28.9 (C-2'), 40.6 (C-3), 43.2 (C-9), 45.3 (C-1''), 52.1 (C-5), 63.9 (C-1'), 68.6 (C-4), 70.5 (2C, C-7, C-8), 72.8 (C-6), 100.9 (C-2), 111.1, 112.0, 115.6, 117.7, 123.4, 127.1, 128.7, 128.9, 132.2, 133.2, 140.9, 148.0 (Ar-C), 170.7, 173.8, 175.3 (3CO).

1-(5-nitro-1H-indole-3-yl-methyl)-4-{sodium [5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-*O*-yl]}-butyl-[1,2,3]-triazole: (SA4NI1)
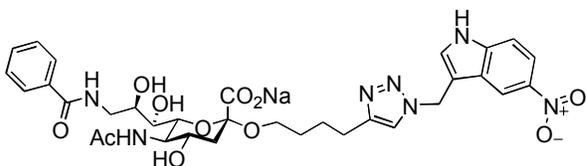
To a solution of **31d** (15.0 mg, 29.0 μmol) and **39** (6.30 mg, 29.0 μmol) in *tert*-BuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.2 mg, 6.0 μmol, 6 μl of freshly prepared 1M solution in H$_2$O), followed by CuSO$_4$·5H$_2$O (0.15 mg, 0.60 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which period at intervals was added the five additional portions of Na-ascorbate (6.0 mg, 30.0 μmol) and three additional portions of CuSO$_4$.5H$_2$O (0.45 mg, 1.83 μmol). After 60 h, according to TLC the reaction was not complete. So a catalytic amount of CuCl$_2$ was added and stirring continued for 1 h after which TLC showed completion of the reaction. The solvents were removed under high vaccum and the residue was purified by reversed phase chromatography on LiCroPrep® RP-8 (5% gradient of MeOH:H$_2$O). After P2 size exclusion chromatography and lyophilization from water, **SA4NI1** (13.0 mg, 61%) was obtained as a white solid.

$[\alpha]_D^{25}$ = - 0.50 (*c* = 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.15-1.26 (m, 2H, H-3'), 1.29 (m, 2H, H-2'), 1.60 (t, J = 12.3 Hz, 1H, H-3a), 2.02 (s, 3H, NHAc), 2.25 (m, 2H, H-4'), 2.70 (dd, J = 4.5, 12.2 Hz, 1H, H-3e), 3.24-3.33 (m, 2H, H-9a, H-1'a), 3.51 (m, 2H, H-7, H-1'b), 3.60-3.76 (m, 3H, H-4, H-6, H-9b), 3.81 (q, J = 10.1 Hz, 1H, H-5), 3.95 (dt, J = 2.2, 8.8 Hz, 1H, H-8), 5.26 (s, 2H, H-1''), 6.99 (t, J = 7.6 Hz, 1H, Ar-H), 7.05 (d, J = 8.8 Hz, 1H, Ar-H), 7.10 (t, J = 7.3 Hz, 1H, Ar-H), 7.21

(s, 1H, Ar-H), 7.34 (d, J = 7.7 Hz, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.44 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.82 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 22.4 (NHCOC$\underline{H}_3$), 24.3 (C-4'), 25.1 (C-3'), 28.7 (C-2'), 40.9 (C-3), 43.3 (C-9), 45.2 (C-1''), 52.2 (C-5), 64.7 (C-1'), 68.7 (C-4), 70.7 (C-7), 70.9 (C-8), 72.8 (C-6), 101.0 (C-2), 111.1, 112.1, 115.4, 117.6, 122.7, 125.1, 127.0, 128.5, 129.2, 131.9, 133.2, 139.6, 140.7, 148.3 (Ar-C), 170.3, 173.8, 175.3 (3CO).

1-(5-nitro-1H-indole-3-propyl)-4-{sodium [5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-*O*-yl]}-methyl-[1,2,3]-triazole: (SA1NI3)
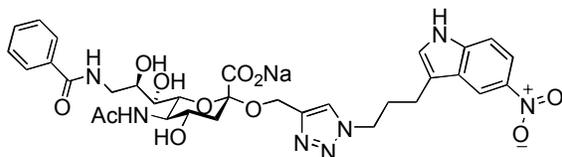
To a solution of **31a** (10.0 mg, 21.0 μmol) and **49** (5.14 mg, 21.0 μmol) in tBuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.2 mg, 6.0 μmol, 6 μl of freshly prepared 1M solution of H$_2$O), followed by CuSO$_4$·5H$_2$O (0.15 mg, 0.60 μmol) in H$_2$O. This heterogeneous mixture was stirred for 36 h during which period at intervals was added four additional portions of Na-ascorbate (4.8 mg, 24.0 μmol in total) and three additional portions of CuSO$_4$·5H$_2$O (0.45 mg, 1.83 μmol in total). After 42 h, according to TLC the reaction was incomplete. So a catalytic amount of CuCl$_2$ was added and stirring continued for 1 h after which TLC showed completion of the reaction. The solvents were removed under high vaccum and the residue was purified by reversed phase chromatography on LiCroPrep® RP-8 (5% gradient of MeOH:H$_2$O) system. After P2 size exclusion chromatography and lyophilization from water, **SA1NI3** (9.0 mg, 60%) was obtained as a white solid.

$[\alpha]_D^{25}$ = - 1.4 (*c* = 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.66 (t, *J* = 12.1 Hz, 1H, H-3a), 1.87 (m, 1H, H-2''), 2.02 (s, 3H, NHAc), 2.16 (m, 2H, H-3''), 2.73 (dd, *J* = 4.6, 12.3 Hz, 1H, H-3e), 3.33 (dd, *J* = 4.0, 8.8 Hz, 1H, H-9a), 3.54 (dd, *J* = 1.4, 8.8 Hz, 1H, H-7), 3.69 (dddd, *J* = 1.9, 4.6, 7.1, 9.8 Hz, 1H, H-4), 3.77-3.87 (m, 3H, H-5, H-6, H-9b), 3.91 (dt, *J* = 2.7, 8.8 Hz, 1H, H-8), 4.14 (t, *J* = 6.3 Hz, 1H, H-

1"), 4.38, 4.65 (A, B of AB, *J* = 12.0 Hz, 2H, H-1'b), 6.80 (s, 1H, Ar-H), 6.98 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.16 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.26 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.45-7.51 (m, 4H, Ar-H), 7.54 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 21.1 (C-3") 22.4 (NHCO$\underline{C}$H$_3$), 29.4 (C-2"), 40.6 (C-3), 43.3 (C-9), 50.1 (C-1"), 52.1 (C-5), 57.4 (C-1'), 68.6 (C-4), 70.6 (C-7), 70.9 (C-8), 73.0 (C-6), 101.0 (C-2), 111.2, 115.7, 116.2, 116.9, 125.2, 125.7, 125.9, 127.1, 128.7, 131.7, 132.9, 139.5, 139.7 (Ar-C), 170.4, 173.4, 175.3 (3CO).

1-(5-nitro-1H-indole-3-propyl)-4-{sodium [5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-*O*-yl]}-ethyl-1,2,3]-triazole: (SA2NI3)
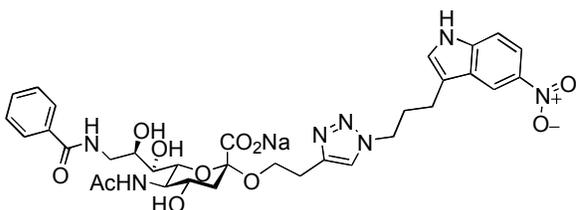
To solution of **31b**(15 mg, 30.0 μmol) and **43** (6.29 mg, 30.0 μmol) in tBuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.2 mg, 6.0 μmol, 6 μl of freshly prepared so1M solution in H$_2$O), followed by CuSO$_4$·5H$_2$O (0.15 mg, 0.60 μmol) in H$_2$O. This heterogeneous mixture was stirred for 42 h during which period at intervals was added five additional portions of Na-ascorbate (6.0 mg, 30.0 μmol) and three additional portions of CuSO$_4$.5H$_2$O (0.45 mg, 1.80 μmol). After 42 h according to TLC the reaction was not complete. So a catalytic amount of CuCl$_2$ was added and stirring continued for 1 h after which TLC showed completion of the reaction. The solvents were removed under high vaccum and purified by reversed phase chromatography on LiCroPrep® RP-8 (5% gradient of MeOH:H$_2$O) system. After P2 size exclusion chromatography and lyophilization from water, **SA2NI3** (5.0 mg, 22%) was obtained as a white solid.

$[\alpha]_D^{25}$ = - 0.1 (*c* = 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.64 (t, *J* = 12.2 Hz, 1H, H-3a), 2.01 (s, 3H, NHAc), 2.04-2.07 (m, 2H, H-2"), 2.45 (m, 2H, H-3"), 2.72 (dd, *J* = 4.5, 12.4 Hz, 1H, H-3e), 2.75-2.80 (m, 2H, H-2'), 3.31 (m, 1H, H-9a), 3.53 (dd, *J* = 1.6, 8.8 Hz, 1H, H-7), 3.60-3.96 (m, 7H, H-4, H-5, H-6, H-8, H-9a, H-1'),

4.15 (t, *J* = 5.9 Hz, 1H, H-1"), 6.80 (s, 1H, Ar-H), 6.93 (s, 1H, Ar-H), 7.18-7.25 (m, 3H, Ar-H), 7.36 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.45-7.49 (m, 3H, Ar-H), 7.72 (dd, *J* = 2.2, 9.0 Hz, 1H, Ar-H), 7.92 (d, *J* = 2.2 Hz, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 21.1 (C-3") 22.4 (NHCO$\underline{C}$H$_3$), 29.4 (C-2"), 40.6 (C-3), 43.3 (C-9), 50.1 (C-1"), 52.1 (C-5), 57.4 (C-1'), 68.6 (C-4), 70.6 (C-7), 70.9 (C-8), 73.0 (C-6), 101.0 (C-2), 111.2, 115.7, 116.2, 116.9, 125.2, 125.7, 125.9, 127.1, 128.7, 131.7, 132.9, 139.5, 139.7 (Ar-C), 170.4, 173.4, 175.3 (3CO).

1-(5-nitro-1H-indole-3-propyl)-4-{sodium [5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-*O*-yl]}-propyl-[1,2,3] triazole: (SA3NI3)
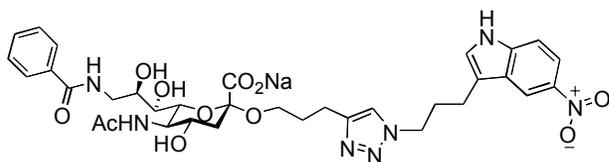
To solution of **31c** (15.0 mg, 30.0 μmol) and **43** (7.35 mg, 30.0 μmol) in tBuOH:H$_2$O (1:1) (0.5 ml) was added Na-ascorbate (1.2 mg, 6.0 μmol, 6 μl of freshly prepared 1M solution in H$_2$O), followed by CuSO$_4$.5H$_2$O (0.15 mg, 0.60 μmol) in H$_2$O. This heterogeneous mixture was stirred for 60 h during which period at intervals was added nine additional portions of Na-ascorbate (10.8 mg, 54.0 μmol) and four additional portions of CuSO$_4$.5H$_2$O (0.60 mg, 2.40 μmol). After 60 h according to TLC the reaction was not complete. So a catalytic amount of CuCl$_2$ was added and stirring continued for 1 h after which TLC showed completion of the reaction. The solvents were removed under high and the residue was purified by reverse chromatography on LiCroPrep® RP-8 (5% gradient of MeOH:H$_2$O). After P2 size exclusion chromatography and lyophilization from water, **SA3NI3** (10.0 mg, 45%) was obtained as a white solid.

$[\alpha]_D^{25}$ = - 0.3 (*c* = 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.66 (m, 3H, H-3a, H-2'), 1.89-2.01 (m, 2H, H-2"), 2.00 (s, 3H, NHAc), 2.36 (t, J = 7.0 Hz, H-3"), 2.40-2.54 (m, 2H, H-3'), 2.72 (dd, *J* = 4.7, 12.5 Hz, 1H, H-3e), 3.36 (m, 2H, H-9a, H-1'a), 3.51 (d, *J* = 8.9 Hz, 1H, H-7), 3.60-3.71 (m, 4H, H-4, H-6, H-9b, H-1'b), 3.84 (m, 2H, H-5, H-8), 4.07 (t, *J* = 6.5 Hz, 1H, H-1"), 6.92 (s, 1H, Ar-H), 7.14 (d, *J* = 8.9

Hz, 1H, Ar-H), 7.22 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.26 (s, 1H, Ar-H), 7.33 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.50 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.6 (dd, *J* = 1.8, 9.1 Hz, 1H, Ar-H), 7.86 (d, *J* = 1.8 Hz, 1H, Ar-H). **^{13}C NMR** (125 MHz, D_2O): δ 22.1 (C-3''), 22.2 (C-3'), 23.2 (NHCO$\underline{C}$H$_3$), 29. 6 (C-2'), 30.2 (C-2''), 41.6 (C-3), 44.2 (C-9), 50.8 (C-1''), 53.1 (C-5), 64.8 (C-1'), 69.5 (C-4), 71.5 (C-7), 71.6 (C-8), 73.7 (C-6), 101.8 (C-2), 112.4, 116.9, 117.2, 118.0, 124.1, 126.9, 127.0, 127.9, 128.0, 129.7, 133.1, 134.3, 140.6 140.9, 148.3 (Ar-C), 171.5, 174.8, 176.3 (3CO).

1-(3-propyl-5-nitro indole)-4{sodium [5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-*O*-yl]-butyl}-1H-[1,2,3] triazole: (SA4NI3)
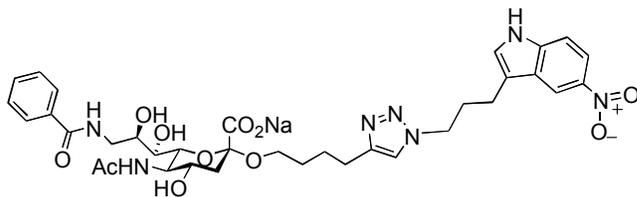
To solution of **31d** (15 mg, 29.0 μmol) and **43** (7.10 mg, 29.0 μmol) in tBuOH:H_2O (1:1) (0.5 ml) was added Na-ascorbate (1.2 mg, 6.00 μmol, 6 μl of freshly prepared solution of 1M in H_2O), followed by CuSO4.5H_2O (0.15 mg, 0.60 μmol) in H_2O. This heterogeneous mixture was stirred for 60 h during which at intervals was added the 9 portions of Na-ascorbate (10.8 mg, 54.0 μmol) and 4 portions of CuSO$_4$.5H_2O (0.60 mg, 2.40 μmol). After 60 h according to TLC the reaction showed slight presence of starting material still. So a catalytic amount of CuCl$_2$ was added and stirred for 1 h after which TLC completion of reaction. Solvents removed under high vaccum to dryness. This was taken in H_2O and purified by reverse chromatography on LiCroPrep® RP-8 bed in MeOH:H_2O (5% gradient) system. After P2 size exclusion chromatography and lyophilization, a white solid of **SA4NI3** (10 mg, 45%) was obtained.

$[\alpha]_D^{25}$ = - 0.7 (*c* = 0.1, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.24 (m, 2H, H-3'), 1.36 (m, 2H, H-2'), 1.66 (t, *J* = 12.1 Hz, 3H, H-3a), 1.89 (m, 2H, H-2''), 2.02 (s, 3H, NHAc), 2.27 (m, 4H, H-4', H-3''), 2.73 (m, 1H, H-3e), 3.31 (m, 2H, H-9a, H-1'a), 3.54 (d, *J* = 8.8 Hz, 1H, H-7), 3.60 (m, 1H, H-1'b), 3.67 (m, 1H, H-4), 3.75-3.85 (m, 3H, H-5, H-6, H-9b), 3.99 (m, 2H, H-8, H-1''), 6.87 (s, 1H, Ar-H), 6.92 (s, 1H,

Ar-H), 7.01, 7.09 (m, 4H, Ar-H), 7.26 (s, 1H, Ar-H), 7.37 (d, J = 2.1 Hz, 2H, Ar-H), 7.54 (d, J = 8.9 Hz, 1H, Ar-H), 7.65 (s, 1H, Ar-H). ^{13}**C NMR** (125 MHz, D_2O): δ 19.6 (C-3''), 20.6 (NHCO$\underline{C}$H$_3$), 22.5 (C-4'), 23.3 (C-3'), 27.1 (C-2'), 27.6 (C-2''), 39.1 (C-3), 41.6 (C-9), 48.1 (C-1''), 50.5 (C-5), 63.0 (C-1'), 66.9 (C-4), 69.0 (C-7), 69.2 (C-8), 71.1 (C-6), 99.3 (C-2), 109.8, 114.0, 114.5, 115.2, 120.7, 124.1, 124.4, 125.2, 126.8, 130.1, 131.5, 137.8, 138.2, 146.1 (Ar-C), 168.3, 172.0, 173.6 (3CO).

1-Pivaloyl-4{sodium (5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosynate-2-*O*-yl)-methyl}-[1,2,3]-triazole: (41)
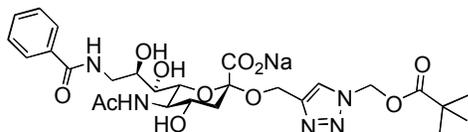
To solution of **31a** (10.0 mg, 21.0 μmol) and **40** (3.92 mg, 25.0 μmol) in tBuOH:H_2O (1:1) (0.5 ml) was added Na-ascorbate (0.41 mg, 2.1 μmol), followed by CuSO$_4$·5H$_2$O (0.052 mg, 0.21 μmol) in H_2O. This heterogeneous mixture was stirred for 48 h during which period at intervals was added three additional portions of Na-ascorbate (1.23 mg, 6.30 μmol) and two additional portions of CuSO$_4$·5H$_2$O (0.104 mg, 0.42 μmol). After 48 h according to TLC the reaction was not complete. So a catalytic amount of CuCl$_2$ was added and stirring continued for 24 h after which TLC showed completion of the reaction. The solvents were removed under high and the residue was purified by reverse chromatography on LiCroPrep® RP-8 (5% gradient of MeOH:H_2O). After P2 size exclusion chromatography and lyophilization from water, **41** (7.0 mg, 45%) was obtained as a white solid.

^{1}H NMR (500 MHz, D_2O): δ 1.11 (s, 9H, C(CH$_3$)$_3$, 1.68 (t, J = 12.2 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.73 (dd, J = 4.6, 12.4 Hz, 1H, H-3e), 3.51-3.55 (m, 2H, H-7, H-9a), 3.66-3.71 (m, 1H, H-4), 3.77-3.86 (m, 3H, H-5, H-6, H-9b), 3.93-3.97 (m, 1H, H-8), 4.60, 4.87 (A, B of AB, J = 12.2 Hz, 2H, H-1'), 6.27 (s, 2H, H-1''), 7.48-7.51 (m, 2H, Ar-H), 7.57-7.60 (m, 1H, Ar-H), 7.74-7.76 (m, 2H, Ar-H), 8.15 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D_2O): δ 22.3 (NHCO$\underline{C}$H$_3$), 26.2 [C($\underline{C}$H$_3$)$_3$], 40.6 (C-3), 43.1 (C-9), , 52.2 (C-5), 57.6 (C-1'), 68.5 (C-4), 70.2 (C-7), 70.5 (C-8), 70.6 (C-

1''), 73.0 (C-6), 101.1 (C-2), 126.4, 127.4, 129.1, 132.4, 133.9, 144.7 (Ar-C), 171.5, 173.5, 175.3 (3CO).

4-methyl-(5-acetamido-9-benzamido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranoside-2-*O*-yl)-[1,2,3]-triazole: (42)
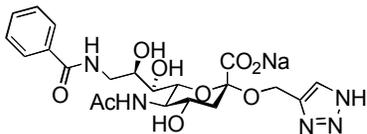
$[\alpha]_D^{25}$ = - 0.9 (*c* = 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O): δ 1.70 (t, *J* = 12.2 Hz, 1H, H-3a), 1.98 (s, 3H, NHAc), 2.73 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3e), 3.53-3.59 (m, 2H, H-7, H-9a), 3.67-3.72 (m, 1H, H-4), 3.75-3.85 (m, 3H, H-5, H-6, H-9b), 3.96 (m, 1H, H-8), 4.67, 4.92 (A, B of AB, *J* = 12.2 Hz, 2H, H-1'), 7.49-7.52 (m, 2H, Ar-H), 7.59 (m, 1H, Ar-H), 7.75-7.77 (m, 2H, Ar-H), 7.89 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, D$_2$O): δ 23.6 (NHCO$\underline{C}$H$_3$), 41.9 (C-3), 44.2 (C-9), 53.4 (C-5), 60.3 (C-1'), 69.7 (C-4), 71.4 (C-7), 71.6 (C-8), 74.2 (C-6), 102.4 (C-2), 128.7, 130.3, 133.7, 135.2 (Ar-C), 173.5, 174.8, 176.2 (3CO).

General procedure for in-situ click chemistry experiments:

The stock solutions of alkynes (10 mM in H$_2$O) and azides (40 mM in DMSO) were prepared. Each alkyne (4 μl) was added to the stock solution of MAG (94 μl of 1 mg/ml solution, in PBS buffer) in an eppendorff. After 2 min the azide compound (2 μl) was added. It was observed that the azide compound did not dissolve completely in the reaction mixture. So the 5 μl of DMSO was added to reaction mixture to dissolve the azide compound. Then the eppendorff was shaken at 37°C for 3 days. So the final concentrations were: MAG (8.77 μM), Alkyne (380 μM) and azide (760 μM).

To cross check the results in parallel two control experiments were also performed, one with BSA (94 μl of 1 mg/ml in PBS buffer) and other in 'absence

of protein'. In the case of 'absence of protein' only PBS buffer with pH 7.4 was used.

LC-MS analysis:

The samples were analyzed by reverse phase chromatography with ESI mode spectroscopic detection in the negative ion mode with selected ion monitoring of only the expected m/z. Samples of the reactions were directly injected (10 µl) into the LC-MS instrument to perform LC-MS/SIM analysis. The products were identified by their retention times and molecular weights. The components were eluted using four different elution solvents: Solvent A1 (0.2% HCOOH in H_2O), A2 (H_2O), B1 (0.2% HCOOH in MeCN) and B2 (MeCN). The presence of 0.2% of HCOOH results in fast denaturation of the protein and release of product on the column. This allows the detection of compound at relatively low concentrations which is an advantage of the LC-MS/SIM analysis.

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Publications:

1) International Patent **WO2005030786 (A1).**

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    3'-N-SUBSTITUTED-3-O-SUBSTITUTED ERYTHRONOLIDE-A DERIVATIVES.

2) **Sachin V. Shelke**, Ganpan Gao, Heiko Gaithe, Sorge Kelm, Oliver Schwardt, Beat Ernst. Synthesis of sialic acid derivatives as ligands for the myelin-associated glycoprotein (MAG), Bioorg Med Chem. 2007 Jul 15;15(14):4951-65.

3) **Sachin V. Shelke**, Brian Cutting, Daniel Strasser, Oliver Schwardt, Beat Ernst* Identification of a Potent Antagonist of MAG by a Dynamic Integrated Approach, **Angewandte Chemie Int. Ed.** (*In manuscript preparation*)

4) Brian Cutting, **Sachin V. Shelke**, Zorica Dragic, Beatrice Wagner, Heiko Gathje, Soerge Kelm and Beat Ernst*. Sensitivity Enhancement in Saturation Transfer Difference (STD) Experiments Through Optimized Excitation Schemes, **Journal of Magnetic Resonance**, (*Submitted* **2006)**.

5) Daniel Strasser, Biran Cutting, **Sachin V. Shelke**, Michele Porro, Oliver Schwardt and Beat Ernst, "A Structural Elucidation and Kinetic Studies of Binding of Sialic acid Ligands to MAG" **Journal of Medicinal Chemistry**, (*In manuscript preparation*)

Poster Presentation and Workshop:

a) My work was presented as an oral abstract at **IUPAC Symposium**, Christchurch, New Zealand by Dr. Laxmi Adhikari on Green Chemistry.

b) Poster presented at European Carbohydrate Symposium, Grenoble, **2003** 'Exploring Carbohydrate Binding site of MAG'.

c) Poster presented at Swiss Chemical Society, Fall Meeting, Lausanne **2003** 'Exploring Carbohydrate Binding Site of MAG'.

d) Poster presented at Swiss Chemical Society, Fall Meeting, Zurich **2004** 'Synthetic Sialic Acid Derivatives as MAG Antagonists'.

e) Poster presenting at Swiss Chemical Society, Fall Meeting, Lausanne **2005** 'Exploring the Binding Site of MAG'.

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Extra-curricular achievments:

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