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Mimetics of the tri- and tetrasaccharide epitope of GQ1bα as myelin-associated glycoprotein (MAG) ligands

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Abstract—The synthesis of phenoxyphenyl, phenoxybenzyl, biphenyl, and phenyltriazole substituted sialic acid derivatives as mimics of the tri- and tetrasaccharide epitopes of GQ1bα is described. These synthetically easily available sialosides show comparable or even enhanced affinity to MAG compared with the natural tri- and tetrasaccharide epitopes and form a new class of potential MAG antagonists.

1. Introduction

To date, three inhibitor proteins of axonal regeneration in the CNS, the myelin-associated glycoprotein (MAG),[1] together with Nogo[2] and OMgp,[3] have been identified. They all bind to the Nogo receptor NgR,[3,4] which interacts with $p75^{NTR}$ to transduce the inhibitory signal across the membrane of a neuron. There, the RhoA-ROCK cascade is activated, which finally leads to growth cone collapse.[5]

In addition, MAG also binds to brain gangliosides, such as GD1a, GT1b, and GQ1bα. The MAG-ganglioside complex interacts with the same coreceptor $p75^{NTR}$ as the NgR complexes to initiate the inhibitory cascade.[6] Therefore, molecules that specifically block the binding of gangliosides to MAG may enhance neurite outgrowth and functional recovery after CNS injury. Structure–activity relationship (SAR) studies of a number of glycosphingolipids indicated that the branched tetrasaccharide moiety Neu5Acα(2-3)Galβ(1-3)[Neu5Acα(2-6)]Gal*N*Ac (**1**) (Fig. 1) of GQ1bα, the ganglioside with the highest affinity to MAG identified so far, represents the minimal binding epitope.[6a] Further SAR studies suggested that the α(2-3)-linked sialic acid on the terminal Gal moiety is the primary determinant for MAG binding,[7] while the additional α(2-6)-linked sialic acid on the Gal*N*Ac moiety only moderately increases the binding affinity.[8] Compared to the contribution of these two sialic acids, the disaccharide core Galβ(1-3)Gal*N*Ac (Fig. 1, in box) behaves more like a spacer to position the two sialic acid moieties in the appropriate spatial orientation needed for binding to MAG.[6a,9]

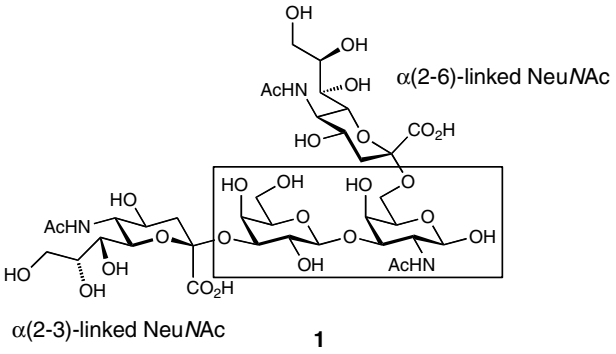

Figure 1. Neu5Acα(2-3)Galβ(1-3)[Neu5Acα(2-6)]Gal*N*Ac (**1**), tetrasaccharide epitope of GQ1bα; the disaccharide core Galβ(1-3)Gal*N*Ac is highlighted in box.

The tetrasaccharide **1** served as the starting point for structural modifications. When Kelm et al.[7c] investigated partial structures thereof, they found that Neu5AcαBn (**2**) has an approximately tenfold higher affinity for MAG than the corresponding α-methyl sialoside **3**, sug-

Keywords: MAG; GQ1bα; Carbohydrate mimetics; Affinity; Suzuki coupling.
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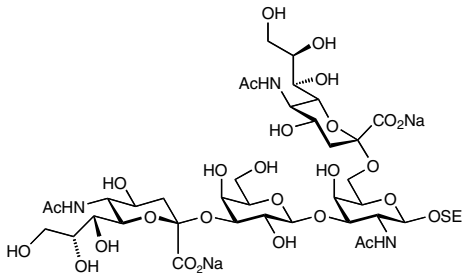
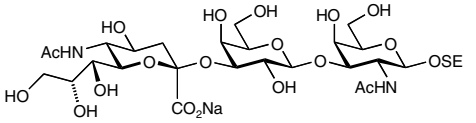
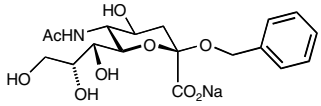
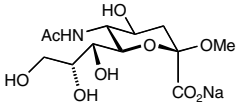
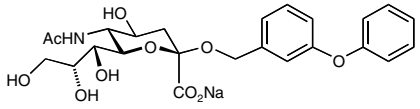
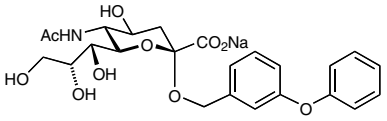
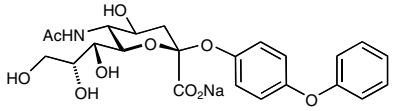
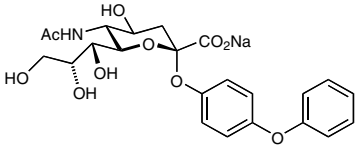
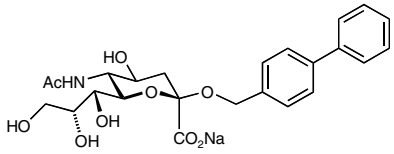
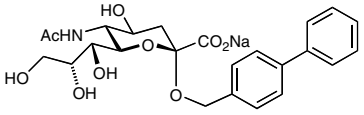
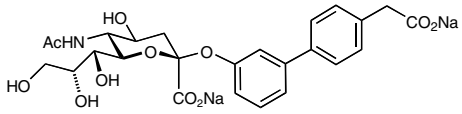
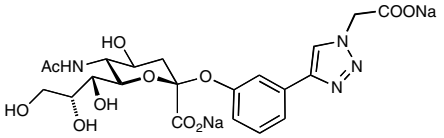
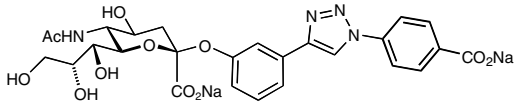
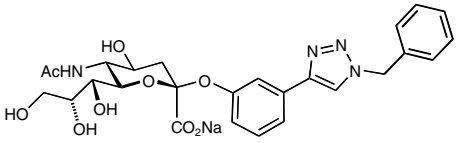
Table 1. Relative inhibitory potencies (rIPs) of sialosides

Structure	rIP	Structure	rIP
1[25] 	4	**30**[24a] 	1
2[7c] 	0.62	**3**[7c] 	0.07
4α 	0.27	**4β** 	n.a.
5α 	0.89	**5β** 	n.a.
6α 	0.38	**6β** 	n.a.
7 	1.0	**27** 	1.1
28 	0.9	**29** 	1.7

The rIP of each sialoside was calculated by dividing the IC_{50} of the reference compound **30** by the IC_{50} of the compound of interest. This results in rIPs above 1.0 for derivatives binding better than **30** and rIPs below 1.0 for compounds with a lower affinity than **30**; n.a., not applicable, less than 50% inhibition at the highest concentration tested (20 mM); OSE = $O(CH_2)_2SiMe_3$.

gesting an additional hydrophobic interaction of the benzyl residue with the receptor site (see Table 1). To further explore such hydrophobic interactions, and to use a hydrophobic replacement of the Galβ(1-3)Gal*N*Ac core to position the carboxylic acid function of the α(2-6)-linked sialic acid in the appropriate spatial position, mimetics of epitope **1** containing aromatic and hetero aromatic cores were synthesized.

2. Results and discussion

Phenoxyphenyl and biphenyl scaffolds are privileged structures frequently found in drugs.[10] It is interesting to note that biphenyl substructures are present in more than 4% of the established drugs from various therapeutic areas.[11] On the search for carbohydrate mimetics, biphenyls were successfully employed to mimic the Galβ(1-4)Glc*N*Ac core in selectin antagonists[12] and the Galβ(1-3)Gal*N*Ac core in MAG antagonists.[13] Therefore, the sialic acid derivatives **4**–**7** (Fig. 2) were designed with phenoxybenzyl, phenoxyphenyl, and biphenyl as reducing end substituents. Whereas **4**–**6** were synthesized to explore the hydrophobic contact suggested by the gain in affinity found for Neu5AcαBn (**2**),[7c] **7** contains an extra acetic acid attached to the biphenyl moiety to mimic the α(2-6)-linked sialic acid of tetrasaccharide **1**.

As shown in Scheme 1, mimetics **4** and **6** were successfully prepared by standard procedures. The glycosylation of 3-phenoxybenzyl alcohol (**8**) or 4-biphenyl methanol (**9**) with the sialyl donor **10**[14] was promoted

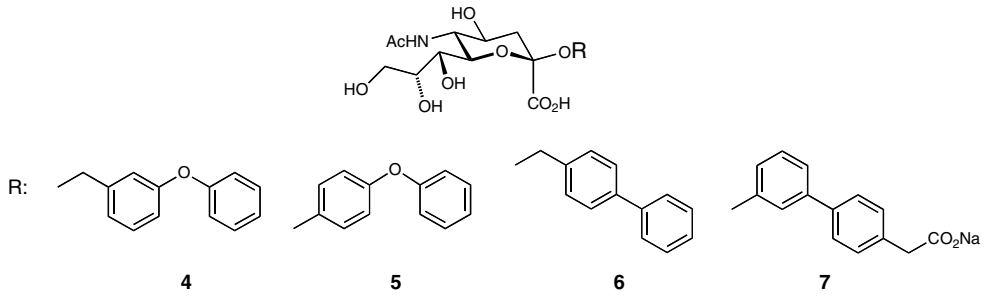
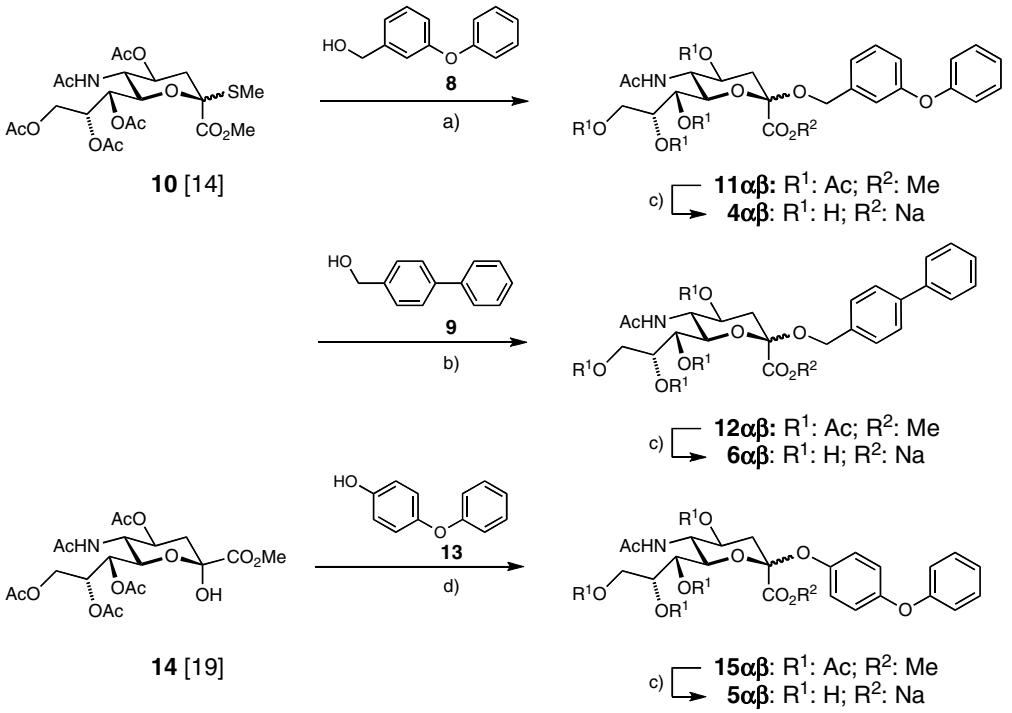


Figure 2. With the sialic acid derivatives **4**-**6**, additional hydrophobic contacts are explored; **7** contains an additional acetic acid attached to the biphenyl moiety to mimic the α(2-6)-linked sialic acid of the tetrasaccharide **1**.

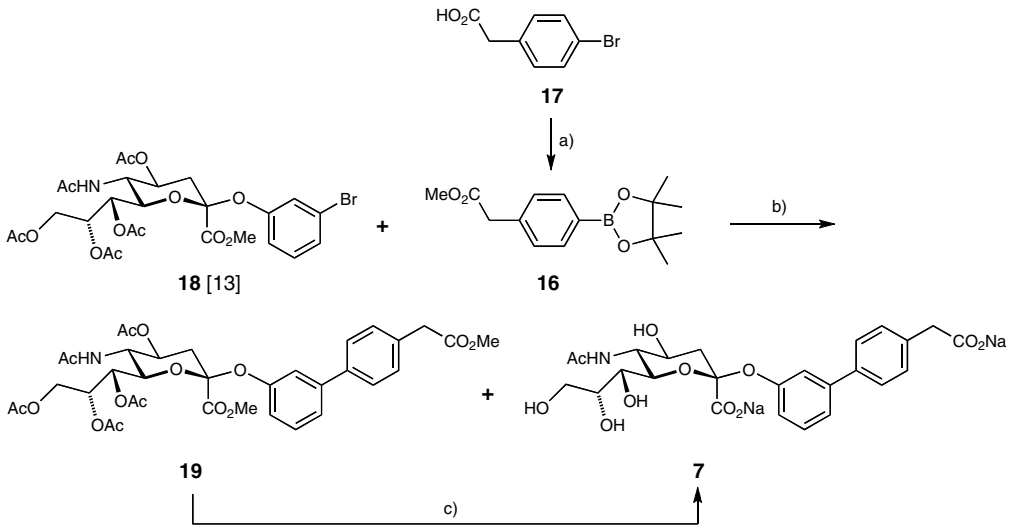


Scheme 1. Reagents and conditions: (a) NIS-TfOH/TMSOTf, MS 3 Å, MeCN, −30 °C, 16 h (81%, α:β = 2.5:1); (b) NIS-TfOH/TMSOTf, MS 3 Å, MeCN, −30 °C, 16 h (88%, α:β = 3:1); (c) NaOMe, MeOH, rt, 4 h; aq NaOH, rt, 16 h (**4α** 60%, **4β** 63%, **5α** 81%, **5β** 95%, **6α** 60%, **6β** 57%); (d) Ph_3P, DEAD, MeCN, 0 °C, 3 h (**15** 75%, α:β = 1.7:1).[18]

by NIS-TfOH[15] or TMSOTf [16] in MeCN at −30 °C. Excellent yields (**11** 81%; **12** 89%) and good stereoselectivities (**11α**:**11β** = 2.5:1; **12α**:**12β** = 3:1) were achieved. After chromatographic separation of the anomers, deprotection under Zemplén conditions followed by hydrolysis in aq NaOH afforded the target compounds **4** and **6** in approx. 60% yields.

However, under identical conditions the sialylation of 4-phenoxyphenol (**13**) was not successful. Using DMTST[17] as promoter in MeCN at 0 °C afforded only 10% of **15** with a reversed stereoselectivity (α:β = 1:2). With NIS-TfOH as promotor, **15** was not formed at all. This failure is most probably due to the low nucleophilicity of phenol **13** compared to the benzyl alcohols **8** and **9**. As previously described,[18] phenol **13** can be sialidated with hemiketal **14**[19] in the presence of Ph_3P and DEAD in MeCN at 0 °C, affording an anomeric mixture of aryl sialoside **15** in good yield (Scheme 1). After chromatographic separation of the anomers followed by hydrolysis, the two diastereomers **5**α and **5**β were obtained.

For the synthesis of mimetic **7**, a Suzuki coupling reaction was applied (Scheme 2). Arylboronic ester **16** was obtained by esterification of 4-bromophenylacetic acid (**17**) in the presence of CAN in MeOH, followed by palladium-catalyzed Miyaura boronation.[20] The slow boronation reaction was decisively accelerated employing microwave radiation (300 W for 1.5 h) leading to the boronic ester **16** in 50% yield. Because the subsequent Suzuki coupling reaction was extremely slow, an elevated reaction temperature was applied, leading to severe decomposition and only traces of the desired product **19**. The reaction between **16** and sialyl derivative **18**[13] (Scheme 2) was substantially accelerated by microwave conditions, leading to the desired product **19**, however, in only 8% yield, along with 3% of **7** (deprotected **19**) and recovered starting material **18** (25%).



Scheme 2. Reagents and conditions: (a) CAN, MeOH, rt, 16 h (90%); bis(pinacolato)diboron, $PdCl_2$ (dppf), dppf, KOAc, dioxane, 120 °C (microwave 300 W), 1.5 h (50%); (b) $PdCl_2$ (dppf), dppf, BHT, K_3PO_4, dioxane, 170 °C (microwave 300 W), 2.25 h (**19** 8%; **7** 3%); (c) NaOMe, MeOH, rt, 4 h; aq NaOH, rt, 16 h (87%).

As a consequence of the low yielding Suzuki coupling reaction, we strived for a suitable replacement of biphenyl-based mimetics. Because triazoles recently attracted broad attention in medicinal chemistry,[21] the corresponding phenyltriazoles were considered as convenient substitutes. In silico investigations supported this approach, since a detailed conformational comparison of the mimetics **7** and **27** with parent compound **1** clearly showed that their low energy conformations are superimposable (Fig. 3). The encircled area indicates that the carboxylic acid substituents in **7** and **27** adopt a similar spatial orientation as the carboxylic acid of the α(2-6)-linked sialic acid in tetrasaccharide **1**. Phenyl-1,2,3-triazoles are accessible by copper-(I)-catalyzed cycloadditions, which recently have drawn broad attention as so-called click chemistry.[21] This modified Huisgen 1,3-dipolar cycloaddition exhibits a number of convincing synthetic advantages such as mild reaction conditions, high yields, and adequate regioselectivity. In addition, triazoles are more than just passive linkers, because they are able to interact with biological targets through hydrogen bonding and dipole interactions.[21]

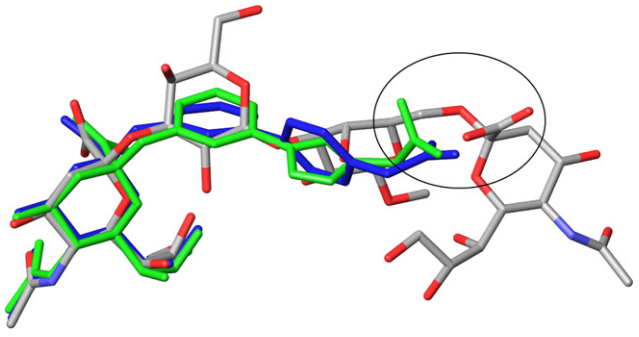
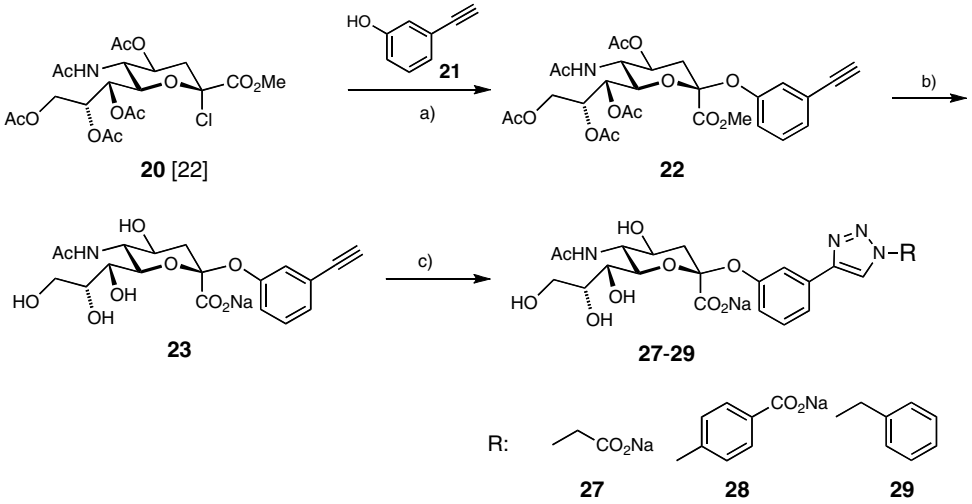


Figure 3. Superimposition of biphenyl and phenyltriazole sialic acid derivatives (**7** and **27**) with the tetrasaccharide **1**. Mimetic **7** is in blue and mimetic **27** in green, while tetrasaccharide **1** is shown in colors according to atom type. The encircled area indicates that the carboxyl group of both synthetic mimics **7** and **27** can reach the region, where a charged interaction of the α(2-6)-linked Neu*N*Ac carboxyl group with the binding site is expected.

The phase transfer reaction with donor **20**[22] and commercially available 3-hydroxyphenylacetylene (**21**) afforded sialyl derivative **22** in 60% yield (Scheme 3). Deprotection by transesterification with NaOMe in MeOH followed by hydrolysis in aq NaOH afforded **23** in 84% yield. The subsequent copper-(I)-catalyzed triazole formations with ethyl azidoacetate (**24**), 4-azidobenzoic acid (**25**), and benzyl azide (**26**) were carried out under standard conditions[21] giving the 1,2,3-triazoles **27**–**29** in approx. 90% yield. It should be noted that the triazole formation starting from the acetate-protected precursor **22** was only partially successful. To force the reaction to completion, the amount of catalyst ($Cu(II)SO_4{\cdot}5H_2O$), from which Cu(I) is generated in situ by sodium ascorbate, had to be increased as much as tenfold. Under these conditions, the desired product was accompanied by the formation of fully and partially deprotected side products. A probable explanation for the different outcome of the cycloaddition reaction is the formation of complexes of Cu(II) with the acetyl protecting groups, thus facilitating their cleavage.

For the inhibition assays, a recombinant protein consisting of the three N-terminal domains of MAG and the Fc part of human IgG (Fc-MAG_{d1-3}) was produced by expression in CHO cells and affinity purification on protein A-agarose.[23] The relative inhibitory potency (rIP) of the sialosides was determined in microtiter plates coated with covalently attached sialic acids as binding target for Fc-MAG_{d1-3}. By complexing the Fc-part with alkaline phosphatase-labeled anti-Fc antibodies and measuring the initial velocity of fluorescein release from fluorescein diphosphate, the amount of bound MAG could be determined. The affinities were measured relative to the reference compound **30**,[24] which has a rIP of 1 (Table 1). Compound **30** was selected as the reference



Scheme 3. Reagents and conditions: (a) $BnEt_3N^+Cl^-$, $CHCl_3$, aq NaOH, reflux, 2 h (60%); (b) NaOMe, MeOH, rt, 2.5 h; aq NaOH, rt, 2.5 h (84%); (c) ethyl azidoacetate (**24**), 4-azidobenzoic acid (**25**), or benzyl azide (**26**), sodium ascorbate (0.1 equiv), $CuSO_4 \cdot 5H_2O$ (0.01 equiv), ^{t}BuOH-H_2O, 16 h (**27**, 93%; **28**, 87%; **29**, 89%).

compound because it represents the minimal carbohydrate epitope required for binding to MAG as identified by Schnaar et al. in their study on gangliosides.[6a] High rIPs represent compounds with high affinity, whereas low rIPs indicate low affinity compounds.

Results from the bioassay confirmed that the disaccharide Galβ(1-3)Gal*N*Ac in the reference compounds **1** and **30** can be replaced by α-linked aromatic aglycons like benzyloxyphenyl (**4α**), phenoxyphenyl (**5α**) or biphenyl (**6α**). The introduction of an additional carboxylate to mimic the acid function of the α(2-6)-linked sialic acid (**6α** → **7**) led to a substantial increase of the affinity to MAG. As expected,[7c] the β-sialosides (**4β**–**6β**) showed no binding affinities, even at the highest concentration tested (20 mM). In addition, phenyltriazole proved to be a valuable replacement of the biphenyl linker, as demonstrated by similar rIPs for **7** and **27**. Moreover, the unexpected high affinity of mimetic **29** (rIP 1.7) compared with **28** (rIP 0.9) implies that instead of the anionic charge, which was supposed to be the key contribution of the α(2-6)-linked sialic acid, a hydrophobic contact in this area of MAG might also contribute to binding.

3. Conclusion

Benzyloxyphenyl, phenoxyphenyl, biphenyl and phenyltriazole based mimetics (**4**–**7**, **27**–**29**) were designed and synthesized. Their affinities to MAG are comparable to the natural carbohydrate ligands **1** and **30**. Because of the pronounced structural simplification combined with the expected improvement of their pharmacokinetic properties,[26] the presented sialosides are the first members of a new generation of MAG antagonists. Especially, the facile, efficient and versatile synthetic accessibility of the phenyltriazole derivatives will allow to further elucidate the structure–affinity relationship of this class of antagonists.

4. Experimental

4.1. General methods

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC). Chemical shifts are expressed in ppm using residual $CHCl_3$ as references. Optical rotations were measured using a Perkin-Elmer Polarimeter 241. ESI-MS analyses were carried out using a Waters Micromass ZQ Detector system. The spectra were recorded in positive ESI mode. HR-MS was measured on a Bruker micrOTOF detector in positive mode. Microwave assisted synthesis was done using a CEM Explorer apparatus. 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 (0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel (Fluka, 40–60 mesh). Methanol (MeOH) was dried by refluxing with sodium methoxide and distilled immediately before use. Dioxane was dried by refluxing with sodium and distilled immediately before use. Acetonitrile (MeCN) and dichloromethane (DCM) were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). Molecular sieves (3 Å) were activated in vacuo at 500 °C for 2 h immediately before use.

4.2. General procedure A: Sialylation of alcohols with methyl 2-thiosialoside 10

To a solution of **10**[14] (1.0 equiv) and alcohol (1.5 equiv) in MeCN was added activated powdered molecular sieves 3 Å. The reaction mixture was stirred at rt under argon for 5 h, then cooled to −30 °C. After addition of NIS (2.0 equiv) and TfOH or TMSOTf (0.2 equiv), stirring was continued for 16 h at −30 °C. Then the mixture was diluted with DCM (20 ml) and filtered through a

pad of Celite. The Celite was washed with DCM (3× 10 ml), and the combined filtrates were washed with 20% aq $Na_2S_2O_3$ (30 ml), 5% aq $KHCO_3$ (2× 20 ml), and H_2O (20 ml), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford α- and β-sialosides as anomeric mixtures.

4.3. General procedure B: Cleavage of *O*-acetyl groups and methyl esters

To a solution of the sialoside in MeOH was added freshly prepared 1 M NaOMe/MeOH. The mixture was stirred at rt for 5 h under argon, then H_2O was added and stirring was continued for 16 h. The solution was neutralized with 0.5% HCl and concentrated. The residue was purified by RP chromatography (5% gradient MeOH in H_2O), ion-exchange chromatography (Dowex 50X8, Na^+ type), and P2 size exclusion chromatography to afford the target molecule after a final lyophilization from H_2O.

4.4. General procedure C: Formation of 1,2,3-triazoles

To a suspension of 3-ethinylphenyl sialoside **23** (1.0 equiv) and azide (1.0 equiv) in *tert*-BuOH/H_2O (1:1) was added sodium ascorbate (0.1 equiv, as freshly prepared 1 M aqueous solution), followed by $CuSO_4{\cdot}5H_2O$ (0.01 equiv, as aqueous solution). The heterogeneous mixture was stirred vigorously at rt for 16 h and then lyophilized. The resulting solid was purified by RP chromatography (5% gradient MeOH in H_2O) to afford the target molecule after a final lyophilization from H_2O.

4.5. Sodium (3′-phenoxybenzyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (4α)

According to general procedure B, **11α** (66.0 mg, 98.0 μmol) in MeOH (4.5 ml) was treated with 1 M NaOMe/MeOH (0.5 ml), followed by H_2O (0.5 ml). **4α** (30.0 mg, 60%) was obtained as a white solid after purification.

$[\alpha]_D$ −27.3 (*c* 0.75, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 1.58 (t, J = 12.1 Hz, 1H, H-3a), 1.94 (s, 3H, NHC-OCH_3), 2.66 (dd, J = 4.4, 12.3 Hz, 1H, H-3b), 3.50 (m, 1H, H-7), 3.52 (dd, J = 5.9, 12.1 Hz, 1H, H-9a), 3.58 (m, 1H, H-4), 3.62 (m, 1H, H-6), 3.66 (m, 1H, H-8), 3.70–3.80 (m, 2H, H-5, H-9b), 4.39, 4.62 (A, B of AB, J = 11.2 Hz, 2H, ArCH_2), 6.91–6.92, 6.97–6.98, 7.09–7.16, 7.26–7.34 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, D_2O): δ 22.4 (NHCOCH_3), 40.8 (C-3), 52.2 (C-5), 62.8 (C-9), 66.9 (ArCH_2), 68.5 (C-7), 68.6 (C-4), 72.0 (C-8), 73.1 (C-6), 101.3 (C-2), 118.8, 119.1, 119.3, 124.1, 124.3, 130.5, 130.6, 139.8, 157.0, 157.2 (12C, C_6H_4, C_6H_5), 173.8, 175.4 (2 CO); HR-MS Calcd for $C_{24}H_{29}NNaO_{10}$ [M+H$^+$]: 514.1689; Found: 514.1687.

4.6. Sodium (3′-phenoxybenzyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (4β)

According to general procedure B, **11β** (25.0 mg, 37.1 μmol) in MeOH (4.5 ml) was treated with 1 M NaOMe/MeOH (0.5 ml), followed by H_2O (0.5 ml). **4β** (12.0 mg, 63%) was obtained as a white solid after purification.

$[\alpha]_D$ +7.8 (*c* 0.62, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.39 (dd, J = 11.6, 13.0 Hz, 1H, H-3a), 1.75 (s, 3H, $NHCOCH_3$), 2.10 (dd, J = 4.9, 13.1 Hz, 1H, H-3b), 3.25 (d, J = 9.6 Hz, 1H, H-6), 3.38 (dd, J = 5.5, 12.0 Hz, 1H, H-9a), 3.53 (dd, J = 2.7, 12.0 Hz, 1H, H-9b), 3.59–3.63 (m, 3H, H-5, H-7, H-8), 3.75 (m, 1H, H-4), 3.98, 4.28 (A, B of AB, J = 10.4 Hz, 2H, ArCH_2), 6.73–6.75, 6.79–6.81, 6.86, 6.91–6.97, 7.11–7.16 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, D_2O): δ 22.3 (NHCOCH_3), 40.8 (C-3), 52.7 (C-5), 64.1 (C-9), 65.1 (ArCH_2), 67.7 (C-4), 68.9 (C-7), 70.6 (C-6), 71.0 (C-8), 100.8 (C-2), 119.1, 119.5, 119.6, 124.6, 124.6, 130.8, 130.9, 140.1, 157.4, 157.6 (12C, C_6H_4, C_6H_5), 175.8 (2C, 2 CO); HR-MS Calcd for $C_{24}H_{29}NNaO_{10}$ [M+H$^+$]: 514.1689; Found: 514.1691.

4.7. Sodium (4′-phenoxyphenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (5α)

According to General procedure B, **15α**[18] (55.9 mg, 84.7 μmol) in MeOH (4.5 ml) was treated with 1 M NaOMe/MeOH (0.5 ml), followed by H_2O (0.5 ml). **5α** (34.1 mg, 81%) was obtained as a white solid after purification.

$[\alpha]_D$ +29.4 (*c* 0.85, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.73 (t, J = 11.9 Hz, 1H, H-3a), 1.85 (s, 3H, NHC-OCH_3), 2.73 (dd, J = 3.6, 12.2 Hz, 1H, H-3b), 3.41–3.45 (m, 2H, H-7, H-9a), 3.56 (m, 1H, H-4), 3.61–3.74 (m, 4H, H-5, H-6, H-8, H-9b), 6.78–6.80, 6.84–6.85, 6.97–6.99, 7.19–7.22 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, D_2O): δ 22.2 (NHCOCH_3), 40.8 (C-3), 51.7 (C-5), 62.2 (C-9), 68.3 (2C, C-4, C-7), 72.1 (C-8), 73.4 (C-6), 103.0 (C-2), 118.5, 119.8, 123.5, 123.7, 130.2, 149.7, 153.3, 157.2 (12C, C_6H_4, C_6H_5), 172.6, 175.2 (2 CO); HR-MS Calcd for $C_{23}H_{26}NNa_2O_{10}$ [M+Na$^+$]: 522.1352; Found: 522.1351.

4.8. Sodium (4′-phenoxyphenyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (5β)

According to general procedure B, **15β**[18] (14.8 mg, 22.4 μmol) in MeOH (4.5 ml) was treated with 1 M NaOMe/MeOH (0.5 ml), followed by H_2O (0.5 ml). **5β** (10.6 mg, 95%) was obtained as a white solid after purification.

$[\alpha]_D$ −45.5 (*c* 0.73, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.65 (dd, J = 11.5, 13.0 Hz, 1H, H-3a), 1.89 (s, 3H, $NHCOCH_3$), 2.41 (dd, J = 4.9, 13.1 Hz, 1H, H-3b), 3.31 (d, J = 9.3 Hz, 1H, H-7), 3.44 (dd, J = 5.5, 11.8 Hz, 1H, H-9a), 3.55 (m, 1H, H-8), 3.59 (dd, J = 2.6, 11.8 Hz, 1H, H-9b), 3.63 (d, J = 10.5 Hz, 1H, H-6), 3.85 (t, J = 10.3 Hz, 1H, H-5), 4.12 (ddd, J = 4.9, 10.3, 11.2 Hz, 1H, H-4), 6.85, 6.89, 6.92–6.94, 6.99–7.02, 7.23–7.26 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, D_2O): δ 22.5 (NHCOCH_3), 41.0 (C-3), 52.2 (C-5), 63.5 (C-9), 67.2 (C-4), 68.7 (C-7), 72.6 (C-8), 71.5 (C-6), 100.9 (C-2), 118.3, 118.4, 120.7, 122.9,

123.7, 125.7, 126.2, 130.4, 151.0, 151.1, 157.9 (12C, C_6H_4, C_6H_5), 175.1, 175.2 (2 CO); HR-MS Calcd for $C_{23}H_{26}NNa_2O_{10}$ [M+Na$^+$]: 522.1352; Found: 522.1356.

4.9. Sodium (4′-biphenylmethyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (6α)

According to general procedure B, **12α** (51.3 mg, 78.0 μmol) in MeOH (4.5 ml) was treated with 1 M NaOMe/MeOH (0.5 ml), followed by H_2O (0.5 ml). **6α** (23.0 mg, 60%) was obtained as a white solid after purification.

$[\alpha]_D$ −22.4 (*c* 0.74, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.42 (t, J = 12.1 Hz, 1H, H-3a), 1.74 (s, 3H, NHCOCH$_3$), 2.50 (dd, J = 4.6, 12.4 Hz, 1H, H-3b), 3.30 (dd, J = 1.8, 9.4 Hz, 1H, H-7), 3.32 (dd, J = 5.8, 12.1 Hz, 1H, H-9a), 3.58 (m, 3H, H-4, H-6, H-8), 3.52 (dd, J = 2.3, 12.0 Hz, 1H, H-9b), 3.54 (t, J = 10.1 Hz, 1H, H-5), 4.27, 4.47 (A, B of AB, J = 11.3 Hz, 2H, ArCH_2), 7.11–7.14, 7.17–7.25, 7.36–7.40 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, D_2O): δ 22.4 (NHCO*C*H$_3$), 40.9 (C-3), 52.3 (C-5), 62.8 (C-9), 67.2 (Ar*C*H$_2$), 68.5 (C-7), 68.7 (C-4), 72.0 (C-8), 73.1 (C-6), 101.5 (C-2), 127.3, 127.4, 128.1, 129.5, 129.7, 136.9, 140.6, 140.6 (12C, C_6H_4, C_6H_5), 174.0, 175.1 (2 CO); HR-MS Calcd for $C_{24}H_{29}NNaO_{10}$ [M+H$^+$]: 498.1740; Found: 498.1739.

4.10. Sodium (4′-biphenylmethyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (6β)

According to general procedure B, **12β** (24.5 mg, 37.3 μmol) in MeOH (4.5 ml) was treated with 1 M NaOMe/MeOH (0.5 ml), followed by H_2O (0.5 ml). **6β** (10.5 mg, 57%) was obtained as a white solid after purification.

$[\alpha]_D$ +21.2 (*c* 0.70, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.53 (t, J = 12.3 Hz, 1H, H-3a), 1.87 (s, 3H, NHCOCH$_3$), 2.24 (dd, J = 4.9, 13.2 Hz, 1H, H-3b), 3.41 (d, J = 9.7 Hz, 1H, H-7), 3.51 (dd, J = 5.6, 12.0 Hz, 1H, H-9a), 3.69 (dd, J = 2.3, 12.0 Hz, 1H, H-9b), 3.73–3.80 (m, 2H, H-5, H-8), 3.83 (d, J = 10.5 Hz, 1H, H-6), 3.90 (m, 1H, H-4), 4.15, 4.47 (A, B of AB, J = 10.2 Hz, 2H, ArCH_2), 7.24–7.27, 7.33–7.39, 7.52–7.53 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, D_2O): δ 22.5 (NHCO*C*H$_3$), 40.3 (C-3), 52.4 (C-5), 64.0 (C-9), 65.0 (Ar*C*H$_2$), 67.4 (C-4), 68.6 (C-7), 70.4 (C-8), 70.7 (C-6), 100.5 (C-2), 127.3, 127.5, 128.2, 129.5, 129.8, 136.7, 140.6, 140.7 (12C, C_6H_4, C_6H_5), 175.2, 175.6 (2 CO); HR-MS Calcd for $C_{24}H_{29}NNaO_{10}$ [M+H$^+$]: 498.1740; Found: 498.1740.

4.11. Methyl (3′-phenoxybenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α and β-D-*galacto*-2-nonulopyranosid)onate (11α) and (11β)

According to general procedure A, **10** (86.8 mg, 0.166 mmol) and **8** (43.4 μL, 0.250 mmol) in MeCN (6 ml) were treated with NIS (75.0 mg, 0.332 mmol) and TfOH (3.0 μL, 33.2 μmol). Silica gel chromatography (1% gradient of MeOH in DCM) afforded **11α** (66.0 mg, 58%) and **11β** (26.5 mg, 23%) as white foams.

Compound **11α**: $[\alpha]_D$ +2.5 (*c* 1.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82 (s, 3H, NHCOCH$_3$), 1.95 (m, 1H, H-3a), 1.96, 1.97, 2.06, 2.08 (4s, 12H, 4 OCOCH$_3$), 2.57 (dd, J = 4.6, 12.8 Hz, 1H, H-3b), 3.62 (s, 3H, OCH$_3$), 4.01 (q, J = 10.8 Hz, 1H, H-5), 4.03 (m, 1H, H-9a), 4.07 (dd, J = 2.1, 10.7 Hz, 1H, H-6), 4.22 (dd, J = 2.7, 12.5 Hz, 1H, H-9b), 4.32, 4.73 (A, B of AB, J = 12.2 Hz, 2H, ArCH_2), 4.81 (ddd, J = 4.6, 10.0, 12.3 Hz, 1H, H-4), 5.12 (d, J = 10.8 Hz, 1H, NH), 5.27 (dd, J = 2.1, 8.6 Hz, 1H, H-7), 5.37 (ddd, J = 2.7, 5.5, 8.4 Hz, 1H, H-8), 6.82–7.28 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7, 20.8, 20.8, 21.1 (4 OCO*C*H$_3$), 23.2 (NHCO*C*H$_3$), 38.7 (C-3), 49.4 (C-5), 52.6 (OCH$_3$), 62.3 (C-9), 66.5 (Ar*C*H$_2$), 67.2 (C-7), 68.4 (C-4), 69.0 (C-8), 72.5 (C-6), 98.5 (C-2), 118.1, 118.3, 118.8, 122.6, 123.2, 129.5, 129.7, 139.3, 157.1, 157.2 (12C, C_6H_4, C_6H_5), 168.3, 170.0, 170.1, 170.2, 170.6, 171.0 (6 CO); ESI-MS Calcd for $C_{33}H_{39}NNaO_{14}$ [M+Na$^+$]: 696.2; Found: 696.2.

Compound **11β**: $[\alpha]_D$ −8.1 (*c* 0.82, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.81 (s, 3H, NHCOCH$_3$), 1.86 (m, 1H, H-3a), 1.89, 1.94, 1.95, 2.08 (4s, 12H, 4 OCOCH$_3$), 2.47 (dd, J = 5.0, 13.0 Hz, 1H, H-3b), 3.67 (s, 3H, OCH$_3$), 3.90 (dd, J = 2.2, 10.5 Hz, 1H, H-6), 4.03 (dd, J = 7.6, 12.4 Hz, 1H, H-9a), 4.08 (q, J = 10.4 Hz, 1H, H-5), 4.41, 4.46 (A, B of AB, J = 12.1 Hz, 2H, ArCH_2), 4.74 (dd, J = 2.5, 12.4 Hz, 1H, H-9b), 5.17 (m, 1H, H-8), 5.21 (m, 1H, H-4), 5.26 (d, J = 10.2 Hz, 1H, NH), 5.32 (dd, J = 2.2, 4.3 Hz, 1H, H-7), 6.85–7.30 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.8, 20.9 (4C, 4 OCO*C*H$_3$), 23.1 (NHCO*C*H$_3$), 38.2 (C-3), 48.3 (C-5), 51.7 (OCH$_3$), 62.3 (C-9), 65.3 (Ar*C*H$_2$), 68.2 (C-7), 68.9 (C-4), 71.7, 71.9 (C-6, C-8), 98.5 (C-2), 117.6, 118.1, 119.1, 122.0, 122.4, 129.8, 129.9, 138.7, 157.0, 157.5 (12C, C_6H_4, C_6H_5), 170.3, 170.6, 171.0 (6C, 6 CO); ESI-MS Calcd for $C_{33}H_{39}NNaO_{14}$ [M+Na$^+$]: 696.2; Found: 696.1.

4.12. Methyl (4′-biphenylmethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α and β-D-*galacto*-2-nonulopyranosid)onate (12α) and (12β)

According to general procedure A, **10** (94.4 mg, 0.181 mmol) and **9** (50.0 mg, 0.271 mmol) in MeCN (6 ml) were treated with NIS (81.4 mg, 0.362 mmol) and TMSOTf (6.5 μL, 36.2 μmol). Silica gel chromatography (toluene/AcOEt 2:1) afforded **12α** (75.0 mg, 63%) and **12β** (24.5 mg, 21%) as white foams.

Compound **12α**: $[\alpha]_D$ +5.5 (*c* 0.87, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82, 1.96, 1.98, 2.08, 2.11 (5s, 15H, NHCOCH$_3$, 4 OCOCH$_3$), 1.99 (m, 1H, H-3a), 2.61 (dd, J = 4.6, 12.8 Hz, 1H, H-3b), 3.63 (s, 3H, OCH$_3$), 4.01–4.07 (m, 2H, H-5, H-9a), 4.09 (dd, J = 2.1, 12.7 Hz, 1H, H-6), 4.28 (dd, J = 2.7, 12.4 Hz, 1H, H-9b), 4.40, 4.79 (A, B of AB, J = 12.0 Hz, 1H, ArCH_2), 4.82 (ddd, J = 4.6, 9.9, 12.3 Hz, 1H, H-4), 5.20 (d, J = 9.7 Hz, 1H, NH), 5.29 (dd, J = 2.0, 8.5 Hz,

1H, H-7), 5.37 (ddd, J = 2.7, 5.6, 8.4 Hz, 1H, H-8), 7.26–7.29, 7.33–7.38, 7.49–7.52 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.8, 20.8, 20.9, 21.1, 23.2 ($NHCOCH_3$, 4 $OCOCH_3$), 38.1 (C-3), 49.4 (C-5), 52.7 (OCH_3), 62.4 (C-9), 66.6 ($ArCH_2$), 67.3 (C-7), 68.5 (C-8), 68.1 (C-4), 72.5 (C-6), 98.5 (C-2), 127.0, 127.0, 127.3, 128.3, 128.7, 129.0, 136.1, 140.7, 140.8 (12C, C_6H_4, C_6H_5), 168.4, 170.1, 170.2, 170.3, 170.7, 171.0 (6 CO); ESI-MS Calcd for $C_{33}H_{39}NNaO_{13}$ [M+Na^+]: 680.2; Found: 680.3.

Compound **12β**: $[\alpha]_D$ −15.5 (c 0.80, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.88 (dd, J = 11.6, 13.0 Hz, 1H, H-3a), 1.81, 1.91, 1.95, 1.98, 2.09 (5s, 15H, $NHCOCH_3$, 4 $OCOCH_3$), 2.51 (dd, J = 5.0, 13.0 Hz, 1H, H-3b), 3.68 (s, 3H, OCH_3), 3.96 (dd, J = 2.2, 10.5 Hz, 1H, H-6), 4.06 (dd, J = 7.8, 12.4 Hz, 1H, H-9a), 4.08 (q, J = 10.3 Hz, 1H, H-5), 4.48, 4.53 (A, B of AB, J = 11.9 Hz, 2H, $ArCH_2$), 4.80 (dd, J = 2.5, 12.4 Hz, 1H, H-9b), 5.21–5.28 (m, 2H, H-4, H-8), 5.32 (d, J = 10.2 Hz, 1H, NH), 5.35 (dd, J = 2.2, 4.1 Hz, 1H, H-7), 7.27–7.54 (m, 9H, C_6H_4, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.8, 20.8, 20.9, 20.9, 23.1 (4 $OCOCH_3$, $NHCOCH_3$), 37.4 (C-3), 49.4 (C-5), 52.7 (OCH_3), 60.4 (C-9), 65.6 ($ArCH_2$), 68.4 (C-7), 69.0 (C-8), 71.9 (2C, C-4, C-6), 98.5 (C-2), 127.1, 127.3, 127.4, 128.0, 128.8, 135.6, 140.7, 140.9 (12C, C_6H_4, C_6H_5), 167.4, 170.2, 170.3, 170.6, 170.8, 171.0 (6 CO); ESI-MS Calcd for $C_{33}H_{39}NNaO_{13}$ [M+Na^+]: 680.2; Found: 680.2.

4.13. Methyl 4′-(4,4,5,5-tetramethyl-1,2,3-dioxaborolan-2-yl)-phenylacetate (16)

To a solution of 4-bromophenylacetic acid (**17**) (700 mg, 3.25 mmol) in MeOH (20 ml) was added CAN (2.68 g, 4.88 mmol) at rt. The mixture was stirred for 16 h and evaporated under reduced pressure. The residue was diluted with DCM (50 ml), washed with H_2O (2× 30 ml), and dried (Na_2SO_4). After filtration and concentration methyl 4-bromophenylacetate (660 mg, 90%) was obtained as colorless oil, which was used without purification.

A microwave tube was charged with methyl 4-bromophenylacetate (150 mg, 655 μmol), potassium acetate (193 mg, 1.96 mmol), bis(picanolato)diborane (200 mg, 786 μmol), $PdCl_2$(dppf) (16.0 mg, 19.7 μmol), and dppf (10.9 mg, 19.7 μmol). The tube was closed, evacuated through a needle, and flushed with argon. Dioxane (5 ml) was added with vigorous stirring. The solvent was degassed in an ultrasonic bath for 15 min and flushed with argon for another 5 min. The tube was heated by microwave irradiation to 120 °C for 1.5 h. The solvent was evaporated in vacuo and the residue dissolved in DCM (50 ml), washed with water (2× 50 ml), dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (1% gradient of AcOEt in toluene) to afford **16** (91.6 mg, 50%) as white foam.

^1H NMR (500 MHz, $CDCl_3$): δ 1.33 (s, 12H, 4 CH_3), 3.64 (s, 2H, CH_2Ph), 3.68 (s, 3H, OCH_3), 7.29, 7.77 (AA′, BB′ of AA′BB′, J = 8.0 Hz, 4H, C_6H_4).

4.14. Methyl [4′-(methyl acetate-2-yl)-biphenyl-3-yl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (19) and sodium [4′-(sodium acetate-2-yl)-biphenyl-3-yl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (7)

A microwave tube was charged with **18**[13] (149 mg, 0.230 mmol), BHT (35.5 mg, 0.160 mmol), dry K_3PO_4 (146.5 mg, 0.690 mmol), $PdCl_2$(dppf) (5.60 mg, 6.90 μmol) and dppf (3.80 mg, 6.90 μmol). The tube was closed, evacuated through a needle at 60 °C for 15 min, and flushed with argon. Dioxane (5.0 ml) and **16** (70.0 mg, 0.254 mmol) were added under vigorous stirring. The mixture was then degassed in ultrasonic bath for 20 min, and heated by microwave irradiation to 170 °C for 2.25 h. The solvent was evaporated in vacuo, the residue dissolved in DCM (50 ml) and washed with H_2O (2× 50 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/AcOEt 3:1–1:6) to afford **19** (12.5 mg, 8%) as white foam. The aqueous layer was concentrated in vacuo to give a brown solid, which was stirred with cold MeOH (10 ml). After filtration and concentration under reduced pressure, the residue was subsequently purified by silica gel chromatography (MeOH/H_2O 10:1), RP chromatography (5% gradient MeOH in H_2O), ion-exchange chromatography (Dowex 50X8, Na^+ type), and P2 size exclusion chromatography to afford **7** (4.00 mg, 3%) as a white solid after a final lyophilization from H_2O.

According to general procedure B, **19** was treated with NaOMe/MeOH, followed by H_2O to give **7** in 87% yield.

Compound **19**: $[\alpha]_D$ +11.4 (c 0.67, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.90 (s, 3H, $NHCOCH_3$), 2.01, 2.02, 2.03, 2.11 (4s, 12H, 4 $OCOCH_3$), 2.20 (t, J = 12.6 Hz, 1H, H-3a), 2.69 (dd, J = 4.7, 13.0 Hz, 1H, H-3b), 3.66 (s, 2H, $ArCH_2$), 3.70, 3.71 (2s, 6H, 2 OCH_3), 4.06 (q, J = 10.4 Hz, 1H, H-5), 4.18 (dd, J = 5.5, 12.5 Hz, 1H, H-9a), 4.33–4.36 (m, 2H, H-6, H-9b), 4.99 (ddd, J = 4.7, 10.4, 12.1 Hz, 1H, H-4), 5.24 (d, J = 10.1 Hz, 1H, NH), 5.34 (dd, J = 1.9, 7.7 Hz, 1H, H-7), 5.40 (m, 1H, H-8), 7.08–7.10, 7.29–7.30, 7.33–7.35, 7.54–7.55 (m, 8H, 2 C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 20.8, 20.9, 21.0 (4 $OCOCH_3$), 23.2 ($NHCOCH_3$), 37.6 (C-3), 40.8 ($ArCH_2$), 49.5 (C-5), 52.1 (OCH_3), 62.0 (C-9), 67.5 (C-7), 68.9 (C-4), 69.5 (C-8), 73.4 (C-6), 100.1 (C-2), 119.2, 119.6, 123.0, 127.3, 129.7, 129.7, 133.4, 139.3, 142.0, 153.8 (12C, 2 C_6H_4), 160.1. 170.1, 170.1, 170.3, 170.6, 171.0 (6 CO); ESI-MS Calcd for $C_{35}H_{41}NNaO_{15}$ [M+Na^+]: 738.2; Found: 738.2.

Compound **7**: $[\alpha]_D$ +12.5 (c 0.26, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.96 (t, J = 12.2 Hz, 1H, H-3a), 2.04 (s, 3H, $NHCOCH_3$), 2.86 (dd, J = 4.7, 12.5 Hz, 1H, H-3b), 3.41–3.45 (m, 2H, H-7, H-9a), 3.57 (s, 2H, $ArCH_2$), 3.60–3.65 (m, 2H, H-7, H-9a), 3.75 (ddd, J = 4.7, 9.2, 12.0 Hz, 1H, H-4), 3.80 (dd, J = 2.4, 12.1 Hz, 1H, H-9b), 3.87–3.94 (m, 3H, H-5, H-6, H-8), 7.12–7.14, 7.38, 7.40–7.50, 7.63 (m, 8H, 2 C_6H_4); ^{13}C

NMR (125 MHz, D_2O): δ 22.3 ($NHCOCH_3$), 41.3 (C-3), 44.4 ($ArCH_2$), 52.1 (C-5), 62.9 (C-9), 68.4 (2C, C-4, C-7), 72.3 (C-8), 73.7 (C-6), 103.1 (C-2), 120.1, 120.5, 123.2, 127.3, 130.1, 130.2, 138.3, 141.9, 153.3, 154.6 (12C, 2 C_6H_4); HR-MS Calcd for $C_{25}H_{28}NNa_2O_{11}$ [M+H$^+$]: 564.1458; Found: 564.1456.

4.15. Methyl (3′-ethinylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside)onate (22)

To a solution of methyl 2-deoxy-2-chloro-4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-neuraminidate(**20**)[22] (295 mg, 0.579 mmol) in $CHCl_3$ (10 ml) was added 3-ethinylphenol (**21**) (0.300 ml, 2.89 mmol) dissolved in 0.2 M aq NaOH (10 ml) at rt, followed by benzyltriethylammonium chloride (290 mg, 1.27 mmol). The reaction mixture was stirred vigorously at 100 °C for 2 h, then diluted with DCM (30 ml) and washed with 0.1 M NaOH (2× 20 ml) and water (2× 20 ml). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel chromatography (0.5% gradient of MeOH in DCM) to afford **22** (205 mg, 60%) as white foam.

$[\alpha]_D$ +13.1 (*c* 0.90, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.91 (s, 3H, $NHCOCH_3$), 2.04, 2.05, 2.13, 2.14 (4s, 12H, 4 $OCOCH_3$), 2.21 (m, 1H, H-3a), 2.64 (dd, *J* = 4.7, 13.0 Hz, 1H, H-3b), 3.07 (s, 1H, C≡CH), 3.70 (s, 3H, OCH_3), 4.08 (q, *J* = 10.4 Hz, 1H, H-5), 4.19 (dd, *J* = 4.9, 12.4 Hz, 1H, H-9a), 4.32–4.37 (m, 2H, H-6, H-9b), 4.97 (ddd, *J* = 4.7, 10.5, 11.9 Hz, 1H, H-4), 5.34–5.38 (m, 2H, H-7, H-8), 5.40 (d, *J* = 10.2 Hz, 1H, NH), 7.11–7.28 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 20.6, 20.7, 20.8 (4 $OCOCH_3$), 23.0 ($NHCOCH_3$), 37.4 (C-3), 49.2 (C-5), 52.9 (OCH_3), 61.9 (C-9), 67.2 (C-7), 68.6 (C-4), 69.2 (C-8), 73.2 (C-6), 82.8 (C≡CH), 99.9 (C-2), 121.0, 122.93, 124.1, 127.9, 129.2, 153.10 (7C, C_6H_4, C≡CH), 167.7, 169.9, 169.9, 170.1, 170.4, 170.7 (6 CO); Anal. Calcd for $C_{28}H_{33}NO_{13}$: C, 56.85; H, 5.62; N, 2.37. Found: C, 56.24; H, 5.52; N, 2.37.

4.16. Sodium (3′-ethinylphenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside)onate (23)

According to general procedure B, **22** (45.0 mg, 76.1 μmol) in MeOH (2.0 ml) was treated with 1 M NaOMe/MeOH (0.2 ml), followed by H_2O (0.5 ml). **23** (27.5 mg, 84%) was obtained as a white solid after purification.

$[\alpha]_D$ +24.3 (*c* 0.91, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.92 (t, *J* = 12.2 Hz, 1H, H-3a), 2.03 (s, 3H, $NHCOCH_3$), 2.88 (dd, *J* = 4.7, 12.5 Hz, 1H, H-3b), 3.49 (s, 1H, C≡CH), 3.60 (dd, *J* = 1.5, 9.2 Hz, 1H, H-7), 3.63 (dd, *J* = 6.3, 12.2 Hz, 1H, H-9a), 3.74 (ddd, *J* = 4.7, 9.6, 11.8 Hz, 1H, H-4), 3.84–3.93 (m, 4H, H-5, H-6, H-8, H-9b), 7.18–7.22, 7.31–7.32 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, D_2O): δ 22.4 ($NHCOCH_3$), 41.1 (C-3), 52.1 (C-5), 63.0 (C-9), 68.4, 68.5 (C-4, C-7), 72.3 (C-8), 73.7 (C-6), 78.7 (C≡CH), 103.1 (C-2), 122.6 (C≡CH), 122.9, 125.5, 128.6, 129.9, 153.9 (6C, C_6H_4), 172.8, 175.5 (2 CO); ESI-MS Calcd for $C_{19}H_{23}NNaO_9$ [M+H$^+$]: 432.1; Found: 432.1.

4.17. Sodium {3′-[1-(sodium acetate-2-yl)-1,2,3-triazol-4-yl]-phenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate (27)

According to general procedure C, **23** (10 mg, 23.2 μmol) and ethyl azidoacetate (**24**) (2.4 μL, 23.2 μmol) in *tert*-BuOH-H_2O (0.4 ml, 1:1) were treated with sodium ascorbate (2.3 μmol, 2.3 μL of a 1 M aqueous solution) and $CuSO_4{\cdot}5H_2O$ (0.06 mg, 0.232 μmol, in 0.8 μL of H_2O) to afford the ethyl ester of **27**, which was then treated with 0.1 M aq NaOH to give **27** (12.0 mg, 93%) after purification.

$[\alpha]_D$ +13.8 (*c* 0.75, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.96 (m, 1H, H-3a), 2.03 (s, 3H, $NHCOCH_3$), 2.92 (m, 1H, H-3b), 3.60–3.62 (m, 2H, H-7, H-9a), 3.75 (m, 1H, H-4), 3.82 (m, 1H, H-9b), 3.89–3.93 (m, 3H, H-5, H-6, H-8), 5.08 (s, 2H, CH_2CO), 7.16 (d, *J* = 8.0 Hz, 1H, C_6H_4), 7.43 (t, *J* = 7.8 Hz, 1H, C_6H_4), 7.60–7.61 (m, 2H, C_6H_4), 8.28 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, D_2O): δ 22.5 ($NHCOCH_3$), 41.4 (C-3), 52.2 (C-5), 63.1 (C-9), 68.6, 68.7 (C-4, C-7), 72.4 (C-8), 73.9 (C-6), 119.2, 121.9, 122.2, 130.6, 147.5, 150.1, 154.8 (8C, C_6H_4, C_2HN_3), 175.5 (3C, 3 CO); HR-MS Calcd for $C_{21}H_{24}N_4Na_3O_{11}$ [M+Na$^+$]: 577.1135; Found: 577.1144.

4.18. Sodium {3′-[1-(sodium benzoate-4-yl)-1,2,3-triazol-4-yl]-phenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate (28)

According to general procedure C, **23** (8.0 mg, 19.0 μmol) and 4-azidobenzoic acid (**25**) (3.0 mg, 19.0 μmol) in *tert*-BuOH-H_2O (0.4 ml, 1:1) were treated with sodium ascorbate (1.9 μmol, 1.9 μL of a 1 M aqueous solution) and $CuSO_4{\cdot}5H_2O$ (0.05 mg, 0.190 μmol, in 0.7 μL of H_2O) to afford **28** (10.2 mg, 87%) as a white solid.

$[\alpha]_D$ +13.9 (*c* 1.16, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.96 (m, 1H, H-3a), 2.04 (s, 3H, $NHCOCH_3$), 2.92 (dd, *J* = 4.6, 12.5 Hz, 1H, H-3b), 3.60–3.64 (m, 2H, H-7, H-9a), 3.74–3.81 (m, 2H, H-4, H-9b), 3.89–3.97 (m, 3H, H-5, H-6, H-8), 7.12–7.14, 7.37–7.40, 7.55–7.58, 7.59, 7.80–7.81, 8.00–8.02, 8.71 (m, 9H, 2 C_6H_4, C_2HN_3); ^{13}C NMR (125 MHz, D_2O): δ 23.4 ($NHCOCH_3$), 41.3 (C-3), 52.1 (C-5), 62.9 (C-9), 68.5, 68.6 (C-4, C-7), 72.3 (C-8), 73.7 (C-6), 103.1 (C-2), 119.0, 120.7, 122.0, 122.0, 130.5, 130.5, 130.7, 138.3, 147.8, 154.6 (14C, 2 C_6H_4, C_2HN_3), 172.9, 175.4 (3C, 3 CO); HR-MS Calcd for $C_{26}H_{27}N_4Na_2O_{11}$ [M+H$^+$]: 617.1472; Found: 617.1475.

4.19. Sodium [3′-(1-benzyl-1,2,3-triazol-4-yl)-phenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (29)

According to general procedure C, **23** (10.0 mg, 23.2 μmol) and benzyl azide (**26**) (2.9 μL, 23.2 μmol) in *tert*-BuOH-H_2O (0.4 ml, 1:1) were treated with sodium ascorbate (2.3 μmol, 2.3 μL of a 1 M aqueous solution)

and $CuSO_4 \cdot 5H_2O$ (0.06 mg, 0.232 μmol, in 0.8 μL of H_2O) to afford **29** (11.7 mg, 89%) as a white solid.

$[\alpha]_D$ +15.6 (*c* 0.58, H_2O); ^1H NMR (500 MHz, D_2O): δ 1.95 (t, $J = 12.2$ Hz, 1H, H-3a), 2.03 (s, 3H, NHC-OCH_3), 2.91 (dd, $J = 4.7$, 12.5 Hz, 1H, H-3b), 3.53 (dd, $J = 5.8$, 12.0 Hz, 1H, H-9a), 3.61 (dd, $J = 1.6$, 9.2 Hz, 1H, H-7), 3.67 (dd, $J = 2.4$, 12.0 Hz, 1H, H-9b), 3.75 (ddd, $J = 4.7$, 9.5, 11.9 Hz, 1H, H-4), 3.82 (ddd, $J = 2.4$, 5.8, 8.7 Hz, 1H, H-8), 3.87 (dd, $J = 1.7$, 10.4 Hz, 1H, H-6), 3.91 (q, $J = 10.4$ Hz, 1H, H-5), 5.56 (s, 2H, PhCH_2), 7.11–7.13, 7.32–7.42, 7.48–7.51 (m, 9H, C_6H_4, C_6H_5), 8.21 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, D_2O): δ 22.2, (NHCOCH_3), 41.0 (C-3), 51.9 (C-5), 54.1 (PhCH_2), 62.6 (C-9), 68.2, 68.3 (C-4, C-7), 72.0 (C-8), 73.5 (C-6), 102.9 (C-2), 118.9, 121.7, 121.7, 122.5, 128.2, 128.9, 129.3, 130.3, 130.6, 135.0, 147.3, 154.5 (14C, C_6H_4, C_6H_5, C_2HN_3), 172.7, 175.2 (2 CO); HR-MS Calcd for $C_{26}H_{30}N_4NaO_9$ $[M+H^+]$: 565.1910; Found: 565.1915.

4.20. Molecular modeling

The low-energy 3D-structures of tetrasaccharide **1** and the synthetic mimetics **7** and **27** were identified in the MacroModel 6.5 modeling environment.[27] Conformation analysis and geometry optimization were performed using the GBSA solvation model and the AMBER force field for carbohydrates published by Still,[28] augmented with parameters derived by Kolb and Ernst.[29] A flexible alignment algorithm implemented in MacroModel 6.5 was used to superimpose synthetic mimics **7** and **27** to tetrasaccharide **1** represented in its global-minimum structure. The best possible overlap of the mimics with the α(2-3)-linked Neu*N*Ac moiety and the carboxyl group of α(2-6)-linked Neu*N*Ac of **1** was achieved by varying torsion angles of five rotatable bonds of the linker between the saccharide subunit and the terminal carboxyl group (Fig. 3). The encircled area indicates that the carboxyl groups of both synthetic mimetics **7** and **27** populate the same region in the 3D-space, where a charged interaction of the carboxyl group of the α(2-6)-linked Neu*N*Ac moiety of **1** with MAG is expected.

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