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Synthesis and Biological Evaluation of Non-Hydrolizable 1,2,3-Triazole Linked Sialic Acid Derivatives as Neuraminidase Inhibitors

Michel Weïwer[a], **Chi-Chang Chen**[a], **Melissa M. Kemp**[c], and **Robert J. Linhardt**[*,a,b,c]

[a] Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, 110, 8th Street, Troy, NY 12180 (USA).

[b] Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110, 8th Street, Troy, NY 12180 (USA).

[c] Department of Biology, Rensselaer Polytechnic Institute, 110, 8th Street, Troy, NY 12180 (USA).

Abstract

α-Sialic acid azide **1** has been used as a substrate for the efficient preparation of 1,2,3-triazole derivatives of sialic acid using the copper-catalyzed azide-alkyne Huisgen cycloaddition ("click chemistry"). Our approach is to generate non-natural *N*-glycosides of sialic acid that are resistant to neuraminidase catalyzed hydrolysis as opposed to the natural *O*-glycosides. These *N*-glycosides would act as neuraminidase inhibitors to prevent the release of new virions. As a preliminary study, a small library of 1,2,3-triazole-linked sialic acid derivatives has been synthesized in 71-89% yield. A disaccharide mimic of sialic acid has also been prepared using the α-sialic acid azide **1** and a C-8 propargyl sialic acid acceptor in 68% yield. A model sialic acid coated dendrimer was also synthesized from a per-propargylated pentaerythritol acceptor. These novel sialic acid derivatives were then evaluated as potential neuraminidase inhibitors using a 96-well plate fluorescence assay; micromolar IC_{50} values were observed, comparable to the known sialidase inhibitor Neu5Ac2en.

Keywords

Sialic acids; Cycloaddition; *N*-glycosides; Neuraminidase inhibitors; Influenza virus

Introduction

Every year, during the flu season, influenza viruses circulate worldwide and are the cause of a great number of fatalities, particularly in young children and the elderly. Influenza viruses are composed of three major classes: influenza virus A, influenza virus B and influenza virus C. Influenza virus A can be subdivided into different serotypes according to the antibody response triggered by these viruses and as many as ten of them have been identified in humans.[1] Among these, H1N1 caused the Spanish flu in 1918,[2] H3N2

[*] Fax: 518-276-3405 linhar@rpi.edu.

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Supporting Information (see footnote on the first page of this article): Spectral data, ^{1}H, ^{13}C NMR of compounds **1, 2a-h, 3, 6-14** and IC_{50} curves of **2a-h, 11, 14**, Neu5Ac2en.

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caused the Hong Kong flu in 1968,[3] and H5N1 (avian influenza A, bird flu) was responsible for the pandemic threat in 2007.[4] From December 2003, through July 2007, 319 human cases of avian influenza A (H5N1) infection were reported, 60% were fatal. All cases reported were from Asia and Africa. These viruses are highly susceptible to mutations, which can also be a threat by decreasing the efficacy of the existing drugs on the market.[5] In this context, there is an urgent need to develop new drugs for the treatment of influenza.

Influenza virus uses their hemagglutinin to bind to sialic acid residues located on the surface of the host cell to gain entry into the cell. Once the cell is infected, the new virions use their neuraminidases to escape the infected cell.[6] Thus, neuraminidases (or sialidases) have been targeted to stop the viral infection by blocking the virus inside the infected cell. Neuraminidase inhibitors have been developed and rapidly introduced to the market. Although M2 protein inhibitors (adamantane structures, amantadine and rimantadine) have also been developed and proven to be efficient against influenza A, they have to be given early in infection and they are ineffective against influenza B. Thus, neuraminidase inhibitors (oseltamivir and zanamivir) are still the preferred drugs for influenza infection. They act as transition-state mimics, inhibiting influenza neuraminidases and preventing new viruses from being released from an infected cell. With only two effective neuraminidase inhibitors on the market, the different forms of influenza viruses have developed resistance through genetic mutations.[7] The wide variety of influenza viruses is a result of their capacity to develop resistance. The small number of effective drugs for the treatment of influenza makes the discovery of new neuraminidase inhibitors a very important goal for increasing the control of known serotypes and to prevent the spread of a new pandemic.

Different analogs of zanamivir have been synthesized to improve efficacy. Modifications of the side chain of sialic acid include the synthesis of dihydropyrancarboxamide derivatives,[8] 7-substituted derivatives,[9] and dimeric conjugates.[10] Recently, the synthesis of 4-triazole-modified zanamivir analogs have been reported by Li *et al.* and led to the discovery of some analogs possessing EC_{50} values comparable to zanamivir.[11] The biologically compatible 1,2,3-triazole group is thus able to replace the guanidine group in the C-4 position of zanamivir without loss of activity.

In our laboratory, we have been focused on the samariumcatalyzed synthesis of non-hydrolyzable *C*-glycosides of sialic acid, like the sTn-KLH, which showed remarkable immunogenicity.[12] These derivatives might be useful in preparing immunogens for active immunization against neuraminic acid containing glycoconjugates in the design and preparation of anti-cancer vaccines with increased biological half-life. In view of the synthetic complexity associated with the preparation of *C*-disaccharides of sialic acid, mainly due to the multi-step synthesis of the sialic acid aldehyde acceptor moiety, we decided to examine the possibility of synthesizing a new class of non-hydrolizable derivatives of sialic acid, *N*-glycosides, using “click chemistry”. Our approach is to develop a simple and efficient strategy that would allow the straightforward synthesis of non-hydrolizable *N*-glycosides of sialic acid as potential neuraminidase inhibitors. The simplicity of the click reaction would ultimately allow the rapid access to a large library of non-hydrolizable *N*-glycosides of sialic acid. The copper-(I)-catalyzed azide-alkyne Huisgen cycloaddition leading to the formation of 1,2,3-triazole derivatives has been widely used during the last decade to develop new pathways to biologically active molecules.[13] It has also been applied successfully in the field of carbohydrate chemistry.[14] We developed a new rapid, high yield, highly regioselective strategy to access *N*-glycosides of sialic acid by reacting the azido sialic acid donor **1** with a variety of different alkynes using the copper-(I)-catalyzed azide-alkyne Huisgen cycloaddition. The sialic acid 1,2,3-triazoles constitute a new class of neuraminidase inhibitors. The availability of a wide variety of alkynes should allow the access to a broad library of this new class of compounds. In this work, a small library of 1,2,3-triazole-linked sialic acids has been synthesized and the strategy has then been applied to the synthesis of a 1,2,3-triazole-linked disaccharide mimic of sialic acid as well as a dendrimeric structure containing four sialic acid residues. The efficiency of the click reaction affords good yields ranging from 68% to 89%. Three of the synthesized compounds were found to have a better IC_{50} than the known neuraminidase inhibitor, Neu5Ac2en, in an *in-vitro* neuraminidase inhibition assay.

Results and Discussion

Development of a clickable sialic acid

In 1991, Tropper and coworkers described the synthesis of the α-sialic acid azide **3** (Scheme 1) starting from the corresponding β-chloride, using a phase transfer catalysis process and NaN_3.[15] We first considered the use of **3** as a potential starting material to synthesize 1,2,3-triazole-linked sialic acids using the copper-(I)-catalyzed azide-alkyne Huisgen cycloaddition (Scheme 1).

3-Butyn-1-ol was used as a model alkyne and different conditions were examined to obtain the 1,2,3-triazole derivative **4**. None of the conditions tested ($CuSO_4$/sodium ascorbate or CuI/DiPEA) provided the desired product and in all cases, the starting material could be completely recovered by extraction. We speculated that the linkage of the azido group to a quaternary carbon might make it too hindered to react with the alkyne. Additionally, stereoelectronic effects from electron withdrawing acetyl protecting groups or an inappropriate conformation of azido sialic acid might not be optimal for reaction with the alkyne. Thus, we decided to change the protecting groups and since the click reaction is compatible with the presence of hydroxyl groups, the unprotected azido sialic acid **1** was initially tested as a potential donor (Scheme 1). Zemplen conditions were used to deprotect **3** affording unprotected azido sialic acid **1**, which was engaged in the click reaction with 3-butyn-1-ol in the presence of copper (II) sulfate and sodium ascorbate as the reducing agent (Scheme 1). The desired 1,2,3-triazole **2**, was obtained in 81% yield and with complete regiospecificity. When the methyl ester protecting group was removed from **1,** prior to the click reaction, to directly afford the fully unprotected target 1,2,3-triazole, the reaction was much slower. Thus, the azido sialic acid donor **1** was selected for subsequent click reactions involving sialic acid.

Synthesis of a small library of 1,2,3-triazole linked sialic acid

A number of alkynes were next used to examine the scope of the click reaction with **1**, and to produce a small library of 1,2,3-triazole derivatives of sialic acid (Table 1). As a preliminary study, we considered diverse alkynes, short, long, rigid and flexible side chains as well as different functional groups, in order to gain some information on the nature of the R group that would favor binding to neuraminidases. The desired 1,2,3-triazole-containing sialic acid compounds were obtained in excellent 71-89% yields and again with complete regiospecificity.

Interestingly, an alkyne substrate containing a conjugated keto group (entry 4) and another one containing a masked aldehyde (entry 7) were clicked and deprotected in excellent yield. The aldehyde-containing compound **2g,** isolated as a mixture of the aldehyde and the corresponding acetal (5:2), was reductively aminated with an amino-protected lysine to convert this mixture to a single compound, **2h**, in 71% yield (Scheme 2). This suggests that aldehyde-containing sialic acid derivatives might find applications for further conjugation *via* reductive amination to surfaces or to KLH-carrier proteins for the production of antibodies for example.

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Synthesis of a 1,2,3-triazole-linked sialic acid disaccharide mimic

We next undertook the synthesis of multivalent 1,2,3-triazole linked sialic acid derivatives in order to increase the affinity of these inhibitors for neuraminidase. A sialic acid disaccharide mimic was first synthesized using the click reaction. This synthesis relied on the azido donor **1** and a C-8 propargyl sialic acid acceptor **10** (Scheme 3). The known sialic acid sulfide **5**[12c] was first protected at the C-8 and C-9 positions as a *p*-methoxybenzylidene, followed by acetylation at C-4 and C-7, to give compound **6** in 86% yield (*exo/endo* ~ 1:1). Regioselective reductive ring opening of the *p*-methoxybenzylidene freed the C-8 position, and compound **7** was obtained in 78% yield. The C-8 hydroxyl group was then propargylated by treatment with propargyl bromide in the presence of barium oxide and barium hydroxide. The C-8 propargyl compound **8** was obtained as the major product in 36% yield as a propargyl ester that lost its C-4 and C-7 acetyl protecting groups due to the presence of barium hydroxide. A mixture of partially deacetylated methyl ester, propargyl ester and carboxylic acid by-products were obtained during this reaction. These by-products were easily converted to the desired compound **9** by acetylation and/or methylation. The acetylation of **8** also allowed us to confirm the position of the propargyl group on the C-8 hydroxyl, along with 2D NMR experiments, because it shifted H-4 and H-7 downfield (see supporting information). Removal of the PMB protecting group using dichlorodicyanoquinone (DDQ) followed by treatment with sodium methoxide in methanol gave the desired C-8 propargyl acceptor **10** in 67% yield.

Using the click conditions previously described, **1** and **10** were coupled and the disaccharide mimic **11** was obtained after demethyl esterification using 0.2 M KOH in 68% yield over 2 steps (Scheme 3).

Synthesis of a model dendrimer of sialic acid

Because of their multivalency leading to higher local concentration, carbohydrate coated dendrimers can show increased activity compared to their corresponding monosaccharides.[16] Roy and coworkers, for example, have shown the potential of sialic acid-coated dendrimers to cross-link and precipitate *Limax flavus* lectin (LFA).[17] We expect that sialic acid coated dendrimers might exhibit enhanced neuraminidase inhibitory activity. We first functionalized pentaerythritol with propargyl groups to obtain a model dendrimer scaffold **12** (Scheme 4). This alkyne containing scaffold was then clicked with the azido sialic acid donor **1**. To facilitate product purification, the reaction mixture was peracetylated with acetic anhydride and pyridine to afford compound **13**. Product **13** was obtained in 32% isolated yield, corresponding to an average 75% yield for the click reaction occurring at each site. Treatment of **13** with a catalytic amount of sodium methoxide in methanol followed by saponification of the methyl ester groups using 0.2 M KOH afforded the fully deprotected dendrimer **14** in 94% yield.

Neuraminidase inhibitor assay

A 96-well plate neuraminidase inhibitor assay, adapted from Gubareva *et al.*,[18] was used to evaluate the biological activity of these novel sialic acid structures. This fluorescence assay measures the release of 4-methylumbelliferone (λ_{ex} 360 nm, λ_{em} 440 nm) produced by the hydrolysis of the substrate (2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid, MUNANA) by the enzyme (neuraminidase from *Clostridium perfringens* (*C. welchii*)) (Scheme 5). *N*-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (Neu5Ac2en), a commercially available and known neuraminidase inhibitor, was used as a positive control.[19] Serial, half-log dilutions (1 mM to 3.16 nM) of each inhibitor were prepared and tested in triplicate in a 96-well plate format and IC_{50} values were calculated. The results are presented in Table 2.

In our assay, Neu5Ac2en (entry 1) was found to have an IC_{50} of 67 μM, which is in the range of literature values.[20] The azido sialic acid (N_3, entry 2), obtained by hydrolysis of **1** using 0.2 M KOH, was found to be a weak neuraminidase inhibitor, with an IC_{50} of 406 μM. When the azido group was replaced by a 1,2,3-triazole group (inhibitors **2a-2h**), the IC_{50} values were variable, depending on the nature of the side chain on the C-4 position of the triazole ring. Although general structure activity relationship of those compounds would require a much larger library, some information can be derived from these results. The presence of a short chain (**2a, b, d, e, f**, entries 3, 4, 6, 7, 8) afforded weak neuraminidase inhibitors having IC_{50} values ranging from 290 μM to > 1 mM. The presence of a longer and more flexible side chain (**2c, h**, entries 5, 10) afforded inhibitors with significantly lower IC_{50}. These results are in agreement with previous studies done in our lab on the synthesis and biological evaluation of *C*-glycoside-type neuraminidase inhibitors.[21] As expected, the disaccharide mimic **11** (entry 11), probably because it can better mimic the non-reducing end of the glycoproteins present on the surface of epithelial cells, and dendrimer **14** (entry 12), due to its multivalency, afforded the lowest IC_{50} values. In order to improve our understanding of the structure-activity relationship of these molecules, a larger library of these novel neuraminidase inhibitors will be prepared as well as higher generations of sialic acid coated dendrimers.

Conclusions

This preliminary study demonstrates that the click reaction can be applied to the synthesis of a library of 1,2,3-triazole derivatives of sialic acid using alkyne-containing molecules. It allows access to multivalent structures of sialic acid like disaccharide analogs and sialic acid coated dendrimers. Screening of a larger library of 1,2,3-triazole derivatives of sialic acid is underway to improve our understanding of the structure-activity relationship of these molecules and to develop a novel class of neuraminidase inhibitors for evaluation as agents for the treatment of influenza. Additional dendrimer scaffolds are also being prepared, with increased valency, as well as sialic acid coated surfaces and materials. The synthesis of an azido sialic acid donor of type **1**, that contains a propargyl group at C-8 or C-9, as a potential building block for the click-based synthesis of polysialic acid analogs is also underway and will be reported in due course. Due to its high efficiency, the click reaction is expected to be transferred to a microarray format for the on-ship synthesis and screening of large libraries of triazole linked sialic acid derivatives, allowing for a better understanding of the structure activity relationships governing their neuraminidase inhibitory effect.

Experimental Section

General methods

Solvents were dried and distilled using classical procedures and stored on molecular sieves 4 Å. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded at room temperature, in $CDCl_3$, MeOD or D_2O (500 MHz). Chemical shifts (δ) are indicated in ppm and coupling constants (*J*) in Hz. ESIMS were recorded on a LC/MSD trap. HRMS were performed on an orbitrap mass spectrometer using electrospray ionization. Thin-layer chromatography (TLC) was carried out using plates of silica gel 60 with fluorescent indicator and revealed with UV light (254 nm) when possible and Von's reagent ($Ce(SO_4)_2$/$(NH_4)_6Mo_7O_{24}.4H_2O/H_2SO_4$). Flash chromatography was performed using silica gel 230-400 mesh.

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Methyl [5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-2-azido-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid]onate 3

Methyl [5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-2-chloro-D-*glycero*-β-D-*galacto*-non-2-ulopyranosid]onate (10.0 mmol, 5.0 g), tetrabutylammonium hydrogenosulfate (10.0 mmol, 3.4 g) and sodium azide (50.0 mmol, 3.26 g) were dissolved in a mixture of CH_2Cl_2/aq.$NaHCO_3$ 1:1 (100 ml) and stirred vigorously for 2 h at room temperature. Dichloromethane was added and the two phases were separated. The organic phase was then washed with saturated aq. $NaHCO_3$, dried over sodium sulfate and filtrated. Evaporation of the solvent under diminished pressure afforded compound 3 as a white solid in 86% yield (4.8 g). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ (ppm): 5.4-5.3 (m, 3H, H-4, H-6, H-7), 5.0 (ddd, 1H, *J* = 11.6 Hz, *J* = 10.3 Hz, *J* = 4.8 Hz, H-8), 4.4 (dd, 1H, *J* = 11.6 Hz, *J* = 3.0 Hz, 1H-9), 4.2-4.1 (m, 2H, H-5, 1H-9), 3.9 (s, 3H, COOMe), 2.6 (dd, 1H, *J* = 13.0 Hz, *J* = 4.8 Hz, H-3eq), 2.15, 2.12, 2.04, 2.03 (4s, $4CH_3COO$), 1.9 (s, 3H, CH_3CON), 1.8-1.7 (m, 1H, H-3ax). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ (ppm): 170.8, 170.6, 170.2, 170.0, 169.9 ($4CH_3\underline{C}OO$, C-1), 167.1 ($CH_3\underline{C}ONH$), 89.0 (C-2), 73.9 (C-6), 69.5 (C-8), 68.8 (C-4), 67.4 (C-7), 62.1 (C-9), 53.5 (C-5), 49.2 ($COO\underline{C}H_3$), 36.5 (C-3), 23.1 ($\underline{C}H_3CONH$), 21.0, 20.8, 20.73, 20.71 ($4\underline{C}H_3COO$). ESIMS *m/z*, calcd for $C_{20}H_{28}N_4O_{12}Na$ $[M+Na]^+$ 539.2, found 539.2.

Methyl [5-acetamido-3,5-dideoxy-2-azido-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid]onate 1

3 (7.66 mmol, 3.95 g) was dissolved in methanol (16 ml) under argon and cooled to 0 °C. A solution of NaOMe/MeOH 0.5 M (7.6 ml) was added dropwise and the reaction was stirred at room temperature for 3 h. After completion of the reaction, Amberlite IR-120 (H^+ form) was added to neutralize the reaction mixture. Evaporation of the solvent under diminished pressure afforded compound 1 as a white solid in quantitative yield (2.7 g). ^{1}H NMR (500 MHz, MeOD, 25 °C) δ (ppm): 3.9 (s, 3H, COOMe), 3.9-3.8 (m, 4H, H-4,5,6 and 1H-9), 3.7 (dd, 1H, *J* = 11.8 Hz, *J* = 5.5 Hz, 1H-9), 3.5 (m, 2H, H-7,8), 2.6 (dd, 1H, *J* = 12.8 Hz, *J* = 4.3 Hz, H-3eq), 2.0 (s, 3H, AcNH), 1.6 (dd, 1H, *J* = 12.8 Hz, *J* = 11.2 Hz, H-3ax). ^{13}C NMR (125 MHz, MeOD, 25 °C) δ (ppm): 175.9 ($CH_3\underline{C}ONH$), 170.5 (C-1), 91.0 (C-2), 76.6 (C-6), 72.4 (C-8), 70.8 (C-7), 68.9 (C-4), 65.5 (C-9), 54.7 (C-5), 54.2 ($COO\underline{C}H_3$), 41.1 (C-3), 23.6 ($\underline{C}H_3CONH$). HRMS *m/z*, calcd for $C_{12}H_{20}N_4O_8Na$ $[M+Na]^+$ 371.1179, found 371.1181.

Typical procedure for the click reaction

In a reaction flask, unprotected azido sialic acid donor 1 (0.2 mmol, 70 mg), alkyne (0.22 mmol), $CuSO_4$ (0.02 mmol, 3.2 mg) and sodium ascorbate (0.1 mmol, 20 mg) were successively added. A mixture of H_2O/t-BuOH/DCM 1/2/1 (4 ml) was then added and the reaction flask was surmounted with a reflux condenser, covered with aluminum foil and warmed to 60 °C. After stirring at this temperature overnight, the mixture was cooled to room temperature and the solvents were evaporated. The mixture was then loaded on a silica gel column and eluted with a gradient of CH_2Cl_2/MeOH 15/1 to 5/1. The corresponding 1,2,3-triazole derivative was obtained and further treated with 0.2 M KOH (1 ml for 0.1 mmol) for 12 h at room temperature. Neutralization with Amberlite IR-120 (H^+ form) followed by filtration and evaporation of the solvents afforded the desired sialic acid derivatives.

5-acetamido-3,5-dideoxy-2-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-D-*glycero*-α-D-*galacto*-non-2-ulopyranosidic acid 2a

$_1$H NMR (500 MHz, D_2O, 25 °C) δ = 7.9 (s, 1H, H-1’), 3.9-3.7 (m, 7H, H-4,5,6,7,8,9), 3.5-3.4 (m, 2H, 2H-4’), 3.1 (dd, 1H, *J* = 13.0 Hz, *J* = 4.5 Hz, H-3eq), 2.8 (t, 2H, *J* = 6.5 Hz, 2H-3’), 2.1 (t, 1H, *J* = 13.0 Hz, H-3ax), 1.9 (s, 3H, AcNH) ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 175.0 (C-1), 170.4 ($CH_3\underline{C}O$), 144.9 (C-2’), 121.8 (C-1’), 90.8 (C-2), 74.2 (C-6), 71.2 (C-8), 68.1 (C-4), 68.0 (C-7), 62.8 (C-9), 60.5 (C-4'), 51.6 (C-5), 39.6 (C-3), 27.8 (C-3'), 22.2 ($\underline{C}H_3CO$) ppm. HRMS *m/z*, calcd for $C_{15}H_{24}N_4O_9$ Na $[M+Na]^+$ 427.1435, found 427.1440.

5-acetamido-3,5-dideoxy-2-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-D-*glycero*-α-D-*galacto*-non-2-ulopyranosidic acid 2b

^{1}H NMR (500 MHz, D_2O, 25 °C) δ = 8.1 (s, 1H, H-1'), 4.6 (s, 2H, 2H-3'), 3.8 (s, 3H, COOMe), 3.8-3.6 (m, 4H, H-4,5,6, 1H-9), 3.5-3.4 (m, 3H, H-7,8, 1H-9), 3.1 (m, 1H, H-3eq), 2.1 (m, 1H, H-3ax), 1.9 (s, 3H, AcNH) ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 174.8 (C-1), 169.3 ($CH_3\underline{C}ON$), 146.4 (C-2'), 121.8 (C-1'), 90.0 (C-2), 74.1 (C-6), 70.6 (C-8), 67.9 (C-4), 67.3 (C-7), 62.7 (C-9), 54.3 (C-3'), 51.3 (C-5), 39.1 (C-3), 21.9 ($\underline{C}H_3CO$) ppm. HRMS *m/z*, calcd for $C_{14}H_{22}N_4O_9Na$ $[M+Na]^+$ 413.1279, found 413.1283.

5-acetamido-3,5-dideoxy-2-(4-(2-octyl-1*H*-1,2,3-triazol-1-yl)-D-*glycero*-α-D-*galacto*-non-2-ulopyranosidic acid 2c

^{1}H NMR (500 MHz, D_2O, 25 °C) δ = 7.9 (s, 1H, H-1'), 3.9-3.4 (m, 7H, H-4,5,6,7,8,9), 3.0 (m, 1H, H-3eq), 2.4 (m, 2H, 2H-3'), 2.1 (m, 1H, H-3ax), 1.9 (s, 3H, AcNH), 1.4 (m, 2H, 2H-4'), 1.0 (broad s, 10H, 10H-5'-9'), 0.6 (t, 3H, *J* = 7.0 Hz, CH_3) ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 174.9 (C-1), 168.6 ($CH_3\underline{C}O$), 147.0 (C-2'), 120.7 (C-1'), 90.0 (C-2), 74.3 (C-6), 70.4 (C-8), 67.7 (C-4), 67.2 (C-7), 62.4 (C-9), 51.4 (C-5), 39.4 (C-3), 31.7, 29.1, 28.6, 24.6, 22.0 ($(\underline{CH_2})_7CH_3$), 22.4 ($\underline{C}H_3CO$), 13.7 ($(CH_2)_7\underline{CH_3}$) ppm. HRMS *m/z*, calcd for $C_{21}H_{36}N_4O_8Na$ $[M+Na]^+$ 495.2425, found 495.2428.

5-acetamido-3,5-dideoxy-2-(4-acetyl-1*H*-1,2,3-triazol-1-yl)-D-*glycero*-α-D-*galacto*-non-2-ulopyranosidic acid 2d

^{1}H NMR (500 MHz, D_2O, 25 °C) δ = 8.7 (s, 1H, H-1'), 3.9-3.8 (m, 3H, H-4,6, 1H-9), 3.8-3.7 (m, 2H, H-5,7), 3.6-3.5 (m, 2H, H-8, 1H-9), 3.2 (dd, 1H, *J* = 12.7 Hz, *J* = 3.9 Hz, H-3eq), 2.5 (s, 3H, 3H-4'), 2.1 (dd, 1H, *J* = 12.7 Hz, *J* = 11.0 Hz, H-3ax), 1.9 (s, 3H, AcNH) ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 194.8 (C-3'), 174.9 (C-1), 169.4 ($CH_3\underline{C}ON$), 146.0 (C-2'), 126.3 (C-1'), 90.6 (C-2), 74.2 (C-6), 70.7 (C-8), 67.9 (C-4), 67.5 (C-7), 62.7 (C-9), 51.3 (C-5), 39.5 (C-3), 26.9 (C-4'), 22.0 ($\underline{C}H_3CO$) ppm. HRMS *m/z*, calcd for $C_{15}H_{21}N_4O_9$ $[M-H]^-$ 401.1314, found 401.1317.

5-acetamido-3,5-dideoxy-2-(4-(1-hydroxycyclopentyl)-1*H*-1,2,3-triazol-1-yl)-D-*glycero*-α-D-*galacto*-non-2-ulopyranosidic acid 2e

^{1}H NMR (500 MHz, D_2O, 25 °C) δ = 8.1 (s, 1H, H-1'), 3.9-3.8 (m, 3H, H-4,6, 1H-9), 3.8-3.7 (m, 2H, H-5,7), 3.5-3.4 (m, 2H, H-8, 1H-9), 3.1 (dd, 1H, *J* = 12.7 Hz, *J* = 3.8 Hz, H-3eq), 2.1 (m, 1H, H-3ax), 2.0-1.8 (m, 4H, 4H-4'), 1.9 (s, 3H, AcNH), 1.8-1.6 (m, 4H, 4H-5') ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 174.8 (C-1), 169.6 ($CH_3\underline{C}O$), 153.1 (C-2'), 119.9 (C-1'), 90.3 (C-2), 78.3 (C-3'), 74.1 (C-6), 70.7 (C-8), 67.9 (C-4), 67.5 (C-7), 62.7 (C-9), 51.3 (C-5), 39.8 (2C-4'), 39.3 (C-3), 22.8 (2C-5'), 21.9 ($\underline{C}H_3CO$) ppm. HRMS *m/z*, calcd for $C_{18}H_{28}N_4O_9Na$ $[M+Na]^+$ 467.1748, found 467.1752.

5-acetamido-3,5-dideoxy-2-(4-((*N*-methylacetamido)methyl)-1*H*-1,2,3-triazol-1-yl)-D-*glycero*-α-D-*galacto*-non-2-ulopyranosidic acid 2f

$_1$H NMR (500 MHz, D_2O, 25 °C) δ = 8.1 and 8.0 (2s, 2H, 2H-1'), 4.52 and 4.45 (2s, 4H, 4H-3'), 3.8 (broad s, 6H, 4H-4,6, 2H-9), 3.7-3.6 (m, 4H, 4H-5,7), 3.5-3.4 (m, 4H, 8H-8, 2H-9), 3.1 (m, 2H, 2H-3eq), 2.9 and 2.7 (2s, 6H, 6H-4'), 2.1 (m, 2H, 2H-3ax), 2.0 and 1.9 (2s, 6H, $2C\underline{H}_3CONMe$), 1.88 and 1.86 (2s, 6H, $2C\underline{H}_3CONH$) ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 174.8 (C-1), 174.1 and 174.0 (2C-5'), 169.2 and 169.1 ($2CH_3\underline{C}ONH$), 143.1 and 142.9 (2C-2'), 121.9 and 121.7 (2C-1'), 89.99 and 89.95 (2C-2), 74.1 (2C-6), 70.5

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(2C-8), 67.9 (2C-4), 67.3 (2C-7), 62.8 (2C-9), 51.3 and 48.7 (2C-5), 45.5 and 42.1 (2C-3'), 39.22 and 39.17 (2C-4'), 36.1 and 33.3 (2C-3), 21.9 and 20.6 (2C-6'), 20.5 and 20.2 ($\underline{C}H_3$CONH) ppm. HRMS *m/z*, calcd for $C_{17}H_{27}N_5O_9Na$ $[M+Na]^+$ 468.1701, found 468.1706.

5-acetamido-3,5-dideoxy-2-(4-formyl-1*H*-1,2,3-triazol-1-yl)-D-*glycero*-α-D-*galacto*-non-2-ulopyranosidic acid 2g

^{1}H NMR (500 MHz, D_2O, 25 °C) δ = 9.9 (s, 1H, CHO), 8.8 (s, 1H, H-1'$_{ald}$), 8.1 (s, 1H, H-1'$_{acetal}$), 6.1 (s, 1H, H-3'$_{acetal}$), 4.0-3.7 (m, 10H, 2H-4,5,6,7, 2H-9), 3.5-3.4 (m, 4H, 2H-8, 2H-9), 3.2 (dd, 1H, *J* = 12.8 Hz, *J* = 3.8 Hz, H-3eq$_{ald}$), 3.1 (dd, 1H, *J* = 13.0 Hz, *J* = 4.0 Hz, H-3eq$_{acetal}$), 2.2-2.1 (m, 2H, H-3ax$_{ald/acetal}$), 1.9 (s, 6H, AcNH$_{ald/acetal}$) ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 186.1 (CHO), 174.9 (C-1), 170.2 ($CH_3\underline{C}O_{acetal}$), 169.9 ($CH_3\underline{C}O_{ald}$), 145.9 (C-2'), 127.2 (C-1'$_{ald}$), 120.6 (C-1'$_{acetal}$), 91.0 (C-2$_{ald}$), 90.6 (C-2$_{acetal}$), 84.7 (C-3'$_{acetal}$), 74.2 (C-6$_{ald}$), 74.1 (C-6$_{acetal}$), 71.0 (C-8$_{acetal}$), 70.96 (C-8$_{ald}$), 67.9 (C-4$_{ald}$), 67.8 (C-4,7$_{acetal}$), 67.7 (C-7$_{ald}$), 62.9 (C-9$_{acetal}$), 62.7 (C-9$_{ald}$), 51.39 (C-5$_{ald}$), 51.35 (C-5$_{acetal}$), 39.6 (C-3$_{ald}$), 39.4 (C-3$_{acetal}$), 22.0 ($\underline{C}H_3$CO) ppm. HRMS *m/z*, calcd for $C_{14}H_{19}N_4O_9$ $[M-H]^-$ 387.1158, found 387.1164.

5-acetamido-3,5-dideoxy-2-(4-((5-(*tert*-butoxycarbonylamino)-5-carboxypentylamino)methyl)-1*H*-1,2,3-triazol-1-yl)-*glycero*-α-D-*galacto*-non-2-ulopyranosidic acid 2h

Compound 2g (21 μmol, 8 mg) and Nα-(*tert*-butoxy-carbonyl)-L-lysine (23 μmol, 6 mg) were dissolved in MeOH/H_2O 1:1 (200 μl) and stirred at room temperature for 30 min. to form the Schiff base. $NaCNBH_3$ (0.3 M in MeOH, 36 μl) was added and the mixture was stirred overnight at room temperature. Solvents were evaporated and the mixture was redissolved in 1 ml of water, loaded on a SAX column and eluted with a gradient of NaCl (0 to 1M). Fractions containing the product were combined and desalted on a P2 biogel to afford 2h in 71% yield (9 mg). ^{1}H NMR (500 MHz, D_2O, 25 °C) δ = 8.2 (s, 1H, H-1'), 4.3 (s, 2H, H-3'), 4.0-3.7 (m, 6H, H-4,5,6,7, 1H-9, H-8'), 3.6-3.5 (m, 2H, H-8, 1H-9), 3.2 (dd, 1H, *J* = 12.8 Hz, *J* = 4.2 Hz, H-3eq), 3.0 (t, 2H, *J* = 7.6 Hz, 2H-4'), 2.1 (dd, 1H, *J* = 12.8 Hz, *J* = 11.2 Hz, H-3ax), 1.9 (s, 3H, AcNH), 1.8-1.6 (m, 4H, 2H-5', 2H-7'), 1.4-1.3 (m, 2H, 2H-6'), 1.3 (s, 9H, Boc) ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 176.9 (C-1), 175.1 (C-5"), 170.5 ($CH_3\underline{C}$ONH), 157.8 (O$\underline{C}$ONH), 138.0 (C-2'), 123.9 (C-1'), 91.0 (C-2), 81.5 (O$\underline{C}Me_3$), 74.2 (C-6), 71.3 (C-8), 68.0, 67.9 (C-4, C-7), 62.8 (C-9), 53.7 (C-5"), 51.5 (C-5), 46.8 (C-3'), 41.3 (C-1"), 39.5 (C-3), 30.1, 29.6 (C-2", C-4"), 27.6 ($C\underline{Me}_3$), 24.9 (C-3"), 22.1 ($\underline{C}H_3$CONH) ppm. HRMS *m/z*, calcd for $C_{25}H_{42}N_6O_{12}Na$$[M+Na]^+$ 641.2753, found 641.2746.

Methyl [Phenyl 5-acetamido-3,5-dideoxy-4,7-di-*O*-acetyl-8,9-(p-methoxybenzylidenyl)-2-thio-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid]onate 6

Neu5Ac phenyl sulfide 5 (2.41 mmol, 1.0 g) was dissolved in MeCN (100 ml), and benzylidene dimethyl acetal (19.2 mmol, 2.9 ml) and camphorsulfonic acid (0.2 mmol, 56 mg) were added. The reaction mixture was stirred for 15 h at room temperature and neutralized with trimethylamine. The solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (100 ml) and washed with saturated aqueous $NaHCO_3$ (60 ml) and water (3 × 25 ml). The organic phase was dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under vacuum. The obtained residue was dissolved in pyridine (5 ml) and acetic anhydride (3 ml) and the mixture was stirred at room temperature overnight. Pyridine and acetic anhydride were evaporated under vacuum and the obtained residue was purified by flash chromatography (petroleum ether/ethyl acetate 1:1 to 0/1) to afford 6 as light yellow foam in 86% yield (1.25 g, *endo/exo* ~1:1). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ = 6.80-7.54 (m, 18H, 10HAr$_{SPh}$, 8HAr$_{PMP}$), 5.5-4.9 (m, 8H, 2CHPh, 2H-4, 2H-6, 2H-7), 4.6-4.0 (m, 8H, 2H-5, 2H-8, 4H-9), 3.83, 3.81 (2s, 6H, 2COOMe), 3.80, 3.6 (2s, 6H, 2PhOMe), 2.8 (m, 1H, 1H-3eq), 2.7 (dd, 1H, *J* = 12.8 Hz, *J* = 4.7 Hz, 1H-3eq), 2.2-1.8 (m, 20H, 4MeCOO, 2MeCONH, 2H-3ax) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ = 171.4, 170.3, 170.2, 170.0, 169.3, 168.54, 168.47, 168.2 (2CH_3CONH, 4CH_3COO, 2$COOCH_3$), 160.3, 159.8 (2CHOMe), 136.84, 136.81, 136.2, 130.2, 130.1, 130.0, 129.6, 129.4, 128.79, 128.76, 128.72, 128.68, 127.83, 127.81, 127.77, 127.6 (12CAr_{SPh}, 6CAr_{PMP}), 113.7, 113.6, 113.4 (4CH_{PMP}), 103.8, 103.4, 101.2 (2CHPh), 87.4, 86.8 (2C-2), 76.0, 75.8, 75.7, 74.8, 73.3, 69.9, 69.5, 69.0, 68.0, 67.8, 66.8, 62.8 (2 × C-4,6,7,8,9), 55.3 (2PhOMe), 52.8, 52.5 (2C-5), 49.5, 49.1 (2COOMe), 37.8, 37.4 (2C-3), 23.4, 23.2 (2CH_3CONH), 21.0, 20.95, 20.85, 20.7 (4OCOMe) ppm. HRMS *m/z*, calcd for $C_{30}H_{35}NO_{11}SNa$ $[M+Na]^+$ 640.1823, found 640.1821.

Methyl [Phenyl 5-acetamido-9-*O*-(*p*-methoxybenzyl)-3,5-dideoxy-4,7-di-*O*-acetyl-2-thio-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid]onate 7

$BH_3 \cdot NH_3$ (5.0 mmol, 365 mg) and $AlCl_3$ (4.86 mmol, 648 mg) were added to a solution of 6 (0.81 mmol, 500 mg) in anhydrous THF (10 ml) with activated molecular sieves 4 Å (2.50 g) at 0 °C. After stirring at 0 °C for 1 h, the reaction mixture was filtered through a pad of celite and the solids were washed with MeCN. The combined filtrate was concentrated and the residue was dissolved in ethyl acetate and washed with saturated aqueous $NaHCO_3$ and water. The organic phase was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 1:1 to 0:1), and afforded 7 as a snow white foam in 78% yield (391 mg). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ = 7.5-7.2 (m, 7H, 5H_{SPh}, 2H_{PMB}), 6.9 (d, 2H, *J* = 8.6 Hz, 2H_{PMB}), 5.9 (d, 1H, *J* = 8.3 Hz, H-6), 5.1 (dt, 1H, J_d = 9.2 Hz, J_t = 2.6 Hz, H-7), 4.9 (ddd, 1H, *J* = 11.6 Hz, *J* = 11.5 Hz, *J* = 4.7 Hz, H-4), 4.5-4.4 (m, 2H, OCH_2Ph), 4.1-4.0 (m, 1H, H-5), 3.9-3.8 (m, 2H, H-8, 1H-9), 3.8 (s, 3H, MeOPh), 3.5 (dd, 1H, *J* = 10.5 Hz, *J* = 1.4 Hz, 1H-9), 3.4 (s, 3H, COOMe), 2.8 (dd, 1H, *J* = 12.8 Hz, *J* = 4.7 Hz, H-3eq), 2.1, 2.0 (2s, 6H, 2OAc), 2.1-2.0 (m, 1H, H-3ax), 1.9 (s, 3H, AcNH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ = 172.4, 171.9, 169.7, 167.6 (COOMe, 2OCOMe, CH_3CONH), 159.0 (C-5'), 135.9, 130.3, 129.5, 129.2, 128.8, 128.7 (6C_{SPh}, 3C_{PMB}), 113.6 (2C-3'), 87.2 (C-2), 75.7 (C-6), 72.8 (OCH_2Ph), 71.3 (C-7), 69.1 (C-4), 67.7 (C-8), 66.3 (C-9), 55.1 (MeOPh), 52.4 (C-5), 51.4 (COOMe), 37.9 (C-3), 23.0 (MeCONH), 21.1 and 20.9 (2MeCOO) ppm. HRMS *m/z*, calcd for $C_{30}H_{37}NO_{11}SNa[M+Na]^+$ 642.1980, found 642.1979.

Propargyl [Phenyl 5-acetamido-9-*O*-(*p*-methoxybenzyl)-3,5-dideoxy-8-*O*-propargyl-2-thio-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid]onate 8

Compound 7 (0.3 mmol, 200 mg), barium oxide (0.91 mmol, 140 mg), and barium hydroxide (0.6 mmol, 188 mg) were dissolved in DMF (10 ml). A solution of propargyl bromide in toluene (80%, 0.32 ml) was then added and the mixture was stirred at room temperature for 10 hours. The reaction was quenched with pTsOH and filtered over a pad of celite. The solids were washed with MeCN, and the filtrates were combined and evaporated. The residue was purified by flash column chromatography (ethyl acetate/methanol 1:0 to 4:1), to afford 8 as a white solid in 36% yield (77 mg). ^{1}H NMR (500 MHz, MeOD, 25 °C) δ = 7.6-7.2 (m, 7H, 5H_{SPh}, 2H_{PMB}), 6.9 (d, *J* = 8.6 Hz, 2H_{PMB}), 5.8 (d, 1H, *J* = 7.4 Hz, H-6), 4.6 (d, 2H, *J* = 2.4 Hz, $COOCH_{2propargyl}$), 4.6-4.5 (m, 2H, OCH_2Ph), 4.3 (dd, 1H, *J* = 16.3 Hz, *J* = 2.4 Hz, 1H-$OCH_{2propargyl}$), 4.2 (dd, 1H, *J* = 16.3 Hz, *J* = 2.3 Hz, 1H-$OCH_{2propargyl}$), 4.0-3.8 (m, 2H, H-4, H-7), 3.8 (s, 3H, MeOPh), 3.8-3.7 (m, 1H, H-5), 3.7-3.6 (m, 1H, 1H-9), 3.6-3.5 (m, 1H, H-8), 3.4 (dd, 1H, *J* = 10.4 Hz, *J* = 1.4 Hz, 1H-9), 3.1-3.0 (m, 2H, H-3eq, 1CCH), 2.5 (d, 1H, *J* = 2.4 Hz, 1CCH), 2.0 (s, 3H, AcNH), 2.0-1.9 (m, 1H, H-3ax) ppm. ^{13}C NMR (125 MHz, MeOD, 25 °C) δ = 172.8 (COOPr), 168.2 (CH_3CONH), 159.0 (C-5'), 136.8, 130.5, 130.3, 129.2, 128.8, 128.1 (6C_{SPh}, 3C_{PMB}), 113.7 (2C-3'), 85.9 (C-2), 79.9 (C-8), 76.9 (C-6), 76.5, 76.0 (2C-$4^{ary}{}_{propargyl}$), 75.3, 74.1 (2$CH_{propargyl}$), 72.8 (OCH_2Ph), 71.1 (C-4), 70.1 (C-7), 68.7 (C-9), 56.3 ($OCH_{2\ propargyl\ ester}$), 55.2 (MeOPh), 53.3 ($OCH_{2\ propargyl\ ether}$), 50.7 (C-5), 36.9 (C-3), 23.1 (MeCONH) ppm. HRMS *m/z*, calcd for $C_{31}H_{35}NO_9SNa[M+Na]^+$ 620.1930, found 620.1938.

Methyl [Phenyl 5-acetamido-9-*O*-(*p*-methoxybenzyl)-3,5-dideoxy-4,7-di-*O*-acetyl-8-*O*-propargyl-2-thio-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid]onate 9

Compound 8 (0.129 mmol , 77 mg) was dissolved in methanol (2 ml) and cooled to 0 °C. A solution of sodium methoxide in methanol (0.5 M, 0.3 ml) was then added and the mixture was stirred for 1 h. The reaction was quenched with Amberlite (H^+ form), washed with methanol and evaporated. The residue obtained was treated with pyridine (1 ml) and acetic anhydride (0.5 ml) at room temperature for 5 h. Evaporation of the volatiles afforded 9 in 95% yield (80 mg). ^{1}H NMR (500 MHz, MeOD, 25 °C) δ = 7.6-7.2 (m, 7H, $5H_{SPh}$, 2H-3”), 6.9 (d, *J* = 8.5 Hz, 2H-4”), 5.5 (m, 1H, H-6), 5.4 (m, 1H, H-4), 5.3 (m, 1H, H-7), 4.5 (d, *J* = 11.5 Hz, 2H-1”), 4.3 (d, *J* = 11.0 Hz, H-5), 4.3-4.1 (m, 2H, 2H-1’), 4.0-3.8 (m, 2H, H-8, 1H-9), 3.8 (s, 4H, H-3’, MeOPh), 3.5 (s, 3H, COOMe), 3.4-3.3 (m, 1H, 1H-9), 3.0 (dd, 1H, *J* = 12.5 Hz, *J* = 4.4 Hz, H-3eq), 2.0 (2s, 6H, 2OAc), 1.96 (s, 3H, AcNH), 1.7 (dd, 1H, *J* = 12.5 Hz, *J* = 11.6 Hz, H-3ax) ppm. ^{13}C NMR (125 MHz, MeOD, 25 °C) δ = 170.6, 170.5, 170.3 (COOMe, 2OCOMe), 168.0 (CH_3CONH), 159.2 (C-5”), 136.2 ($2CH_{SPh}$), 136.0 (C-2”), 129.9 ($4^{ary}C_{SPh}$), 129.6 (2C-3”), 129.0 ($1CH_{SPh}$), 128.8 ($2CH_{SPh}$), 113.7 (2C-4”), 87.5 (C-2), 79.6 (C-2’), 74.6 (C-8), 73.4 (C-6), 72.9 (C-3’), 70.5 (OCH_2Ph), 68.7 (C-4, C-7), 67.7 (C-9), 56.7 (MeOPh), 55.3 (C-5, C-1’), 52.5 (COOMe), 38.2 (C-3), 23.7 (MeCONH), 21.1 and 20.9 (2MeCOO) ppm. HRMS *m/z*, calcd for $C_{33}H_{39}NO_{11}SNa$ $[M+Na]^+$ 680.2136, found 680.2138.

Methyl [Phenyl 5-acetamido-3,5-dideoxy-8-*O*-propargyl-2-thio-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid]onate 10

To a solution of compound 9 (19 μmol, 13 mg) in DCM/H_2O 20:1 (0.5 ml) at 0 °C, DDQ (20 μmol, 5 mg) was added. The reaction mixture was stirred vigorously for 1 hour and poured into a saturated aqueous $NaHCO_3$ solution. The mixture was extracted with DCM followed by drying over Na_2SO_4, filtration and evaporation under reduced pressure. The obtained residue was dissolved in anhydrous methanol (0.5 ml), cooled to 0 °C and sodium methoxide in methanol solution (0.5 M, 0.1 ml) was added. After stirring for 2 h at room temperature, the reaction was quenched with Amberlite (H^+ form), washed with methanol and evaporated. The residue was purified by flash column chromatography (DCM/methanol 15:1 to 7/1), to afford 10 as a white solid in 67% yield (5 mg). ^{1}H NMR (500 MHz, MeOD, 25 °C) δ = 7.6-7.3 (m, 5H, $5H_{SPh}$), 4.3 (s, 2H, 2H-1’), 3.9 (t, 1H, *J* = 10.3 Hz, H-6), 3.9-3.8 (m, 3H, H-4,7, 1H-9), 3.7 (s, 3H, COOMe), 3.7-3.6 (m, 2H, H-5, 1H-9), 3.5-3.4 (m, 2H, H-8,3’), 3.1 (dd, 1H, *J* = 12.8 Hz, *J* = 4.6 Hz, H-3eq), 2.0 (s, 3H, AcNH), 1.8 (dd, 1H, *J* = 12.8 Hz, *J* = 11.3 Hz, H-3ax) ppm. ^{13}C NMR (125 MHz, MeOD, 25 °C) δ = 175.5 (C-1), 171.7 (CH_3CONH), 138.7 ($2CH_{SPh}$), 132.1 (CH_{SPh}), 131.0 ($4^{ary}C_{SPh}$), 130.8 ($2CH_{SPh}$), 88.7 (C-2), 81.0 (C-2’), 78.1 (C-8), 77.1 (C-6), 73.8 (C-3’), 70.9 (C-4), 65.3 (C-7), 58.6 (C-9), 54.2 (C-5), 52.6 (C-1’), 50.7 ($COOCH_3$), 39.8 (C-3), 23.6 (CH_3CONH) ppm. HRMS *m/z*, calcd for $C_{21}H_{27}NO_8SNa$ $[M+Na]^+$ 476.1361, found 476.1352.

Disaccharide mimic 11

In a reaction flask was successively added the unprotected azido sialic acid donor 1 (13 μmol, 4.5 mg), the C-8 propargyl acceptor 10 (11 μmol, 5 mg), $CuSO_4$ (1.2 μmol, 0.2 mg) and sodium ascorbate (6 μmol, 1.2 mg). A mixture of H_2O/t-BuOH/DCM 1/2/1 (0.4 ml) was then added and the reaction flask was covered with aluminum foil and warmed to 60 °C. After stirring at this temperature overnight, the mixture was cooled to room temperature and the solvents were evaporated. The mixture was then loaded on a silica gel column and eluted with a gradient of CH_2Cl_2/MeOH 10:1 to 5:1. The disaccharide intermediate was obtained as a white solid in 68% (6 mg). It was then treated with 0.2 M KOH (0.1 ml) for 12 h at room temperature. Neutralization with Amberlite IR-120 (H^+ form) followed by filtration and evaporation of the solvents afforded the desired sialic acid disaccharide mimic 11 in quantitative yield (5 mg). ^{1}H NMR (500 MHz, D_2O, 25 °C) δ = 8.1 (s, 1H, H-1”), 7.5 (d, 2H, *J* = 7.5 Hz, $2H_{SPh}$), 7.4 (d, 1H, *J* = 7.5 Hz, $1H_{SPh}$), 7.3 (t, 2H, $2H_{SPh}$), 4.7 (d, 1H, *J* = 8.5 Hz, 1H-3”), 4.6 (d, 1H, *J* = 8.5 Hz, 1H-3”), 3.9-3.4 (m, 14H, 2H-4, 2H-5, 2H-6, 2H-7, 2H-8, 4H-9), 3.2 (dd, 1H, *J* = 12.5 Hz, *J* = 4.0 Hz, 1H-3eq), 2.9 (dd, 1H, *J* = 12.9 Hz, *J* = 4.5 Hz, 1H-3eq), 2.1 (dd, 1H, *J* = 12.5 Hz, *J* = 11.5 Hz, 1H-3ax), 1.9, 1.8 (2s, 6H, 2AcNH), 1.9-1.8 (m, 1H, 1H-3ax) ppm. ^{13}C NMR (125 MHz, D_2O, 25 °C) δ = 175.0, 173.7 (2C-1), 171.8, 170.3 ($2CH_3\underline{C}ONH$), 143.6 (C-2”), 136.4, 130.3, 129.0, 127.7, 127.3 ($6C_{SPh}$), 122.9 (C-1”), 90.8, 87.2 (2C-2), 75.9, 75.0, 74.2, 71.9, 71.2, 70.4, 68.1, 66.8 (2C-4, 2C-6, 2C-7, 2C-8), 63.2, 62.6 (2C-9), 61.3 (C-3”), 51.5, 49.8 (2C-5), 38.9, 37.5 (2C-3), 22.1 ($2\underline{C}H_3CONH$) ppm. HRMS *m/z*, calcd for $C_{31}H_{42}N_5O_{16}S$ $[M-H]^-$ 772.2353, found 772.2346.

3-(3-(prop-2-ynyloxy)-2,2-bis((prop-2-ynyloxy)methyl)propoxy)prop-1-yne 12

Pentaerythritol (5 mmol, 681 mg), KOH (76 mmol, 4.25 g) and DMF (15 ml) were stirred at 0 °C for 5 min. An 80% solution of propargyl bromide in toluene (60 mmol, 10 ml) was added dropwise and the mixture was kept at 50 °C overnight. After cooling to room temperature, the mixture was extracted with diethyl ether, dried over Na_2SO_4, filtered and evaporated. After flash chromatography on silica gel (petroleum ether/ethyl acetate, v/v, 1/1 to 0/1), compound 12 was obtained as light yellow syrup in 42% yield (608 mg). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ = 4.1 (s, 8H, 8H-2), 3.5 (s, 8H, 8H-1), 2.4 (s, 4H, 4H-3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ = 80.3 (C-4), 74.4 (C-5), 69.2 (C-2), 58.9 (C-3), 45.0 (C-1) ppm. ESIMS *m/z*, calcd for $C_{17}H_{20}O_4Na$ $[M+Na]^+$ 826.6, found 826.6.

Protected dendrimer 13

In a reaction flask was successively added the unprotected azido sialic acid donor 1 (0.2 mmol, 70 mg), compound 12 (0.032 mmol, 9 mg), $CuSO_4$ (0.02 mmol, 3.2 mg) and sodium ascorbate (0.1 mmol, 20 mg). A mixture of H_2O/t-BuOH/DCM 1/2/1 (4 ml) was then added and the reaction flask was covered with aluminum foil and warmed to 60 °C. After stirring at this temperature overnight, the mixture was cooled to room temperature and the solvents were evaporated. Pyridine (1.5 ml) and acetic anhydride (1 ml) were added and the mixture was stirred for 5 h at room temperature under argon. After evaporation of the volatiles, the mixture was loaded on a silica gel column and eluted with a gradient of ethyl acetate/ methanol 1/0 to 9/1. The corresponding 1,2,3-triazole dendrimer 13 was obtained as a colorless film in 32% yield (24 mg). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ = 8.1 (s, 4H, 4H-1’), 5.5-5.4 (m, 8H, 4H-4 and 4H-6), 5.1 (td, 4H, *J* = 11.4 Hz, *J* = 5.1 Hz, 4H-8), 4.6 (s, 8H, 8H-3’), 4.4 (d, 4H, *J* = 10.7 Hz, 4H-9), 4.3 (d, 4H, *J* = 11.4 Hz, 4H-7), 4.2-4.0 (m, 8H, 4H-9 and 4H-5), 3.8 (s, 12H, 4COOMe), 3.6 (8H, s, 8H-2”), 3.4 (4H, dd, *J* = 13.0 Hz, *J* = 4.3 Hz, 4H-3eq), 2.6 (4H, dd, *J* = 12.6 Hz, *J* = 12.3 Hz, 4H-3ax), 2.2, 2.1, 2.07, 2.01 (48H, 4s, $16CH_3COO$), 1.9 (12H, s, $4CH_3CONH$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ = 174.3 (4C-1), 173.1, 172.6, 172.5, 172.3 ($16CH_3\underline{C}OO$), 168.5 ($4CH_3\underline{C}ONH$), 148.1 (4C-2’), 124.1 (4C-1’), 90.9 (4C-2), 75.7 (4C-6), 71.2 (4C-2”), 71.14 (4C-3’), 71.11 (4C-8), 70.0 (4C-4), 69.0 (4C-7), 66.3 (4C-9), 64.3 (4C-5), 55.6 (4COO$\underline{Me}$), 50.6 (4C-1”), 38.0 (4C-3), 23.6, 22.29, 22.28, 21.8, 21.6 ($20\underline{C}H_3CO$) ppm. HRMS *m/z*, calcd for $C_{97}H_{133}N_{16}O_{52}$ $[M+H]^+$ 2353.8185, found 2353.8189.

Deprotected dendrimer 14

Compound 13 (8.5 μmol, 20 mg) was dissolved in anhydrous methanol (0.1 ml) and cooled to 0 °C. Sodium methoxide in methanol (0.5 M, 0.04 ml) was added dropwise and the reaction mixture was stirred at room temperature for 5 h. After neutralization with Amberlite (H^+ form), filtration and evaporation of the solvents, the partially deprotected dendrimer was treated with 0.2 M KOH (0.5 ml) for 12 h at room temperature. Neutralization with Amberlite (H^+ form) followed by filtration and evaporation of the solvents afforded 14 in 94% yield (13 mg). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ = 8.1 (s, 4H, 4H-1'), 4.4-4.3 (m, 4H, 4H-6), 4.3 (s, 8H, 8H-3'), 3.9 (s, 8H, 8H-2"), 3.8-3.6 (m, 8H, 4H-4, 4H-9), 3.5-3.4 (m, 8H, 4H-5, 4H-7), 3.3-3.2 (m, 8H, 4H-8 and 4H-9), 3.1 (m, 4H, 4H-3eq), 2.1 (dd, 4H, *J* = 12.8 Hz, *J* = 10.9 Hz, 4H-3ax), 1.9 (s, 12H, $4CH_3CONH$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ = 174.8 (4C-1), 169.2 ($4CH_3CONH$), 143.7 (4C-2'), 122.8 (4C-1'), 90.0 (4C-2), 74.1 (4C-6), 70.6 (4C-2"), 67.9 (4C-8), 67.7 (4C-4), 67.4 (4C-7), 63.0 (4C-3'), 62.7 (4C-9), 51.3 (4C-5), 44.3 (4C-1"), 39.2 (4C-3), 22.0 ($4\underline{C}H_3CONH$) ppm. HRMS *m/z*, calcd for $C_{61}H_{93}N_{16}O_{36}$ $[M+H]^+$ 1625.5933, found 1625.5935.

Neuraminidase inhibition assay

4-Methylumbelliferyl-*N*-acetylneuraminic acid (MUNANA), *N*-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (Neu5Ac2en), neuraminidase from *Clostridium perfringens* (*C. welchii*) and sodium acetate were purchased from Sigma and used as received. 96-Well plates, white polystyrene, flat bottom, non-treated, non-sterile, Costar, were from Corning Inc.

The assays were performed at a total volume of 100 μl in a 96-well plate. Serial half-log dilutions of each inhibitor and a commercially available inhibitor, Neu5Ac2en, were prepared from 3.16 nM to 1 mM in 0.1 mM sodium acetate buffer, pH 5.0 in triplicate, to determine IC_{50} of each inhibitor. Neuraminidase (0.5 mU) and different concentrations of the inhibitors were added to each well and allowed to incubate for 30 min at room temperature. The substrate (0.5 mM) was added and the reaction mixture was incubated for 1 hour at 37 °C. Immediately following incubation, the fluorescence was read on a Molecular Devices SpectraMax M5, with an excitation wavelength at 360 nm and an emission wavelength at 440 nm. The percent of neuraminidase activity was plotted against inhibitor concentration to determine the IC_{50} using software from GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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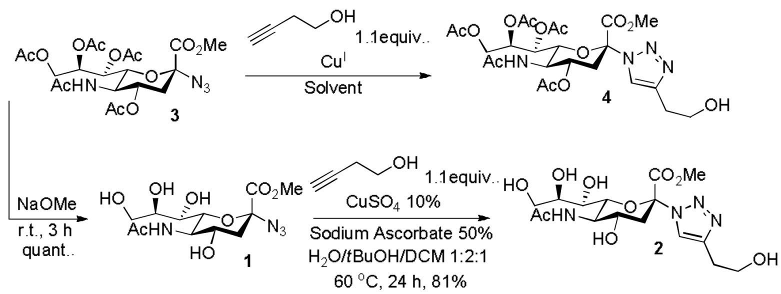

Scheme 1.

Use of a peracetylated (**3**) or unprotected (**1**) α-sialic acid azide donor.

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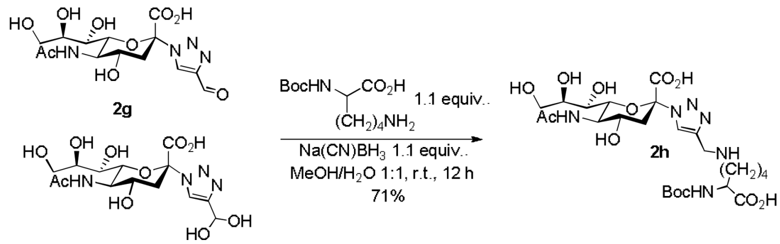
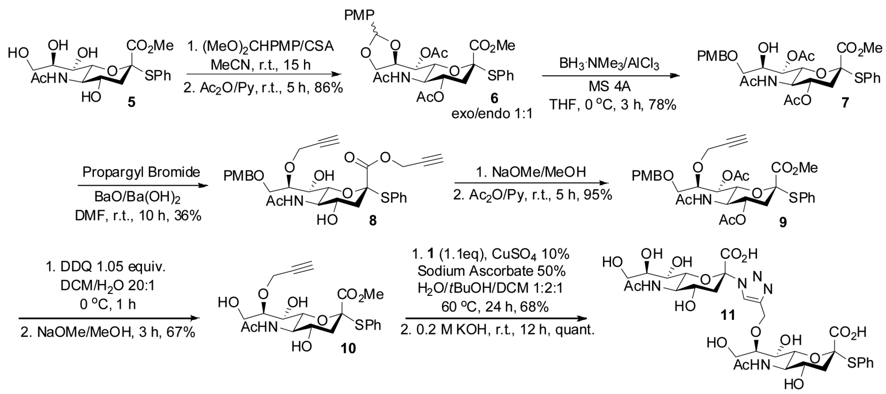


Scheme 2.
Reductive amination of **2g** using a lysine residue.



Scheme 3.

Synthesis of a 1,2,3-triazole-linked disaccharide mimic of sialic acid 11.

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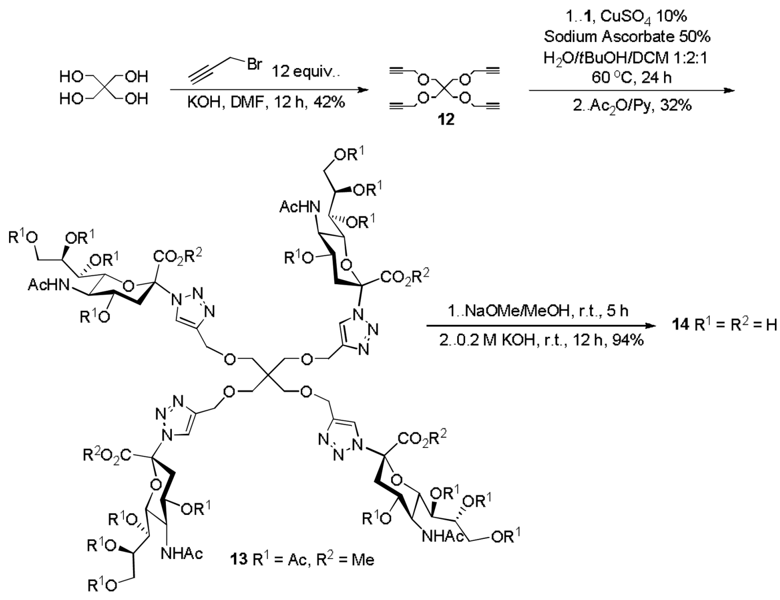
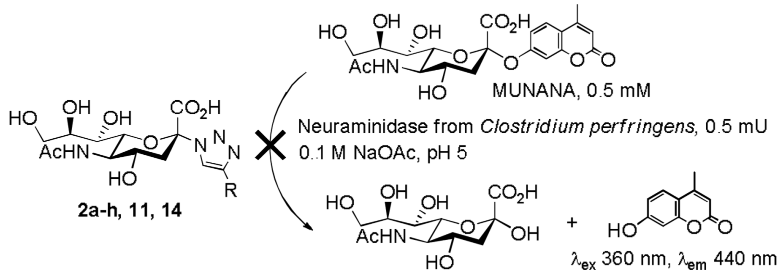


Scheme 4.

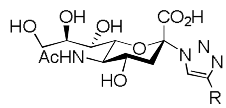
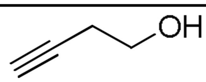
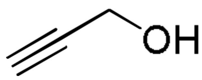
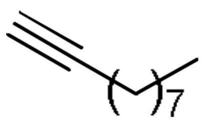
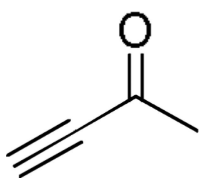
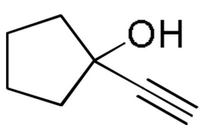
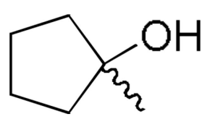
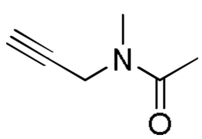
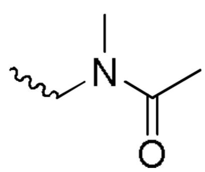
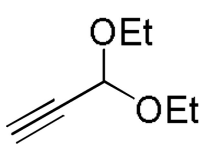
Synthesis of a model dendrimer of sialic acid 14.



Scheme 5.
Neuraminidase inhibitor assay.

Table 1

Synthesis of a small library of 1,2,3-triazole-linked sialic acid.[a]

Entry	Alkyne	Product	Yield
			
1		R = CH_2CH_2OH	2a, 81%
2		CH_2OH	2b, 85%
3		$(CH_2)_7CH_3$	2c, 89%
4		$COCH_3$	2d, 79%
5			2e, 81%
6			2f[b], 86%
7		CHO	2g[c], 71%

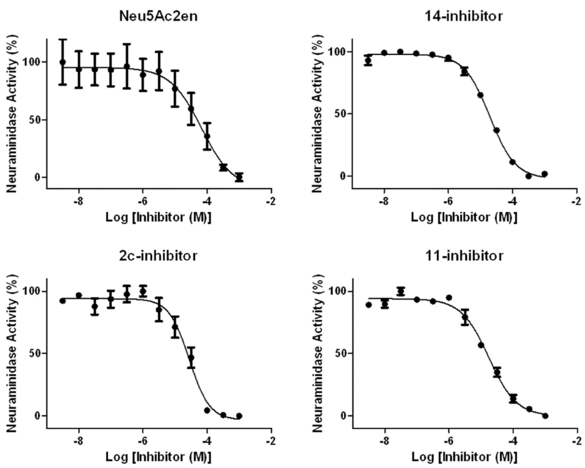
[a]The unprotected azido sialic acid donor **1** (0.2 mmol, 70 mg), the alkyne (0.22 mmol), $CuSO_4$ (0.02 mmol, 3.2 mg), sodium ascorbate (0.1 mmol, 20 mg) in a mixture of H_2O/*t*BuOH/DCM 1:2:1 (4 ml) was covered with aluminum foil and heated to 60 °C overnight. After purification on silica gel, the corresponding 1,2,3-triazole derivatives were obtained and further treated with 0.2 M KOH for 12 h at room temperature.

[b]H and ^{13}C NMR shows the presence of 2 diastereoisomers in a 5:3 ratio, due to the presence of a chiral nitrogen atom.

[c]Sialoside **2g** was isolated as a mixture of aldehyde and the corresponding hydrated acetal in a 5:2 ratio.

Table 2

Evaluation of the IC_{50} values of inhibitors



Entry	Inhibitor	IC_{50} (μM)
1	Neu5Ac2en	67
2	N_3	406
3	2a	290
4	2b	> 1000
5	2c	28
6	2d	549
7	2e	> 1000
8	2f	343
9	2g	-
10	2h	133
11	11	17
12	14	20