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Triazole-Linked Sialic Acids as Neuraminidase Inhibitors

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

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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 perpropargylated pentaerythritol acceptor. These novel sialic acid derivatives were then evaluated as potential neuraminidase inhibitors using a 96-well plate fluorescence assay; micromolar IC50 values were observed, comparable to the known sialidase inhibitor Neu5Ac2en.

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 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 viruses use 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 dihydropryanocarboxamide 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 samarium-catalyzed 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-hydrolyzable 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-hydrolyzable N-glycosides of sialic acid as potential neuraminidase inhibitors. The simplicity of the click reaction would ultimately allow rapid access to a large library of non-hydrolyzable 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 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 co-workers described the synthesis of the $\alpha$-sialic acid azide 3 (Scheme 1) starting from the corresponding $\beta$-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 Cu(II)/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 because the click reaction is compatible with the presence of hydroxy 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 protecting group of the methyl ester 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.

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Alkyne</th>
<th>Product</th>
<th>Yield</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>R-CH₂CH₂OH</td>
<td>2a, 81%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CH₃OH</td>
<td>2b, 85%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(CH₂)₂CH₂</td>
<td>2c, 89%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>COCH₃</td>
<td>2d, 79%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>2e, 81%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>2f, 86%</td>
<td></td>
</tr>
</tbody>
</table>

[a] The unprotected azido sialic acid donor 1 (0.2 mmol, 70 mg), the alkyne (0.22 mmol), CuSO₄ (0.02 mmol, 3.2 mg), sodium ascorbate (0.1 mmol, 20 mg) in a mixture of H₂O/tBuOH/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] ¹H and ¹³C 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.

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 by reductive amination to surfaces or to KLH-carrier proteins for the production of antibodies for example.

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 hydroxy 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 into 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 hydroxy, along with 2D NMR experiments, because it shifted 4-H and 7-H 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 de-methyl esterification using 0.2 m KOH in 68% yield over 2 steps (Scheme 3).

Scheme 2. Reductive amination of 2g using a lysine residue.
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. Roy and co-workers, for example, have shown the potential of sialic acid coated dendrimers to cross-link and precipitate *Limax flavus* lectin (LFA). 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 (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., was used to evaluate the biological activity of these novel sialic acid structures. This fluorescence assay measures the release of 4-methylumbelliferone ($\lambda_{ex} = 360$ nm, $\lambda_{em} = 440$ nm) produced by the hydrolysis...
of the substrate \([2'-(4\text{-methylumbelliferyl})-\alpha-D-N\text{-acetyl}\text{-neuraminic acid, MUNANA}]\) by the enzyme [neuraminidase from \textit{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 \(\mu\)M to 3.16 \(\mu\)M) 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.

Scheme 5. Neuraminidase inhibitor assay.

Table 2. Evaluation of the IC\(_{50}\) values of inhibitors.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Inhibitor</th>
<th>IC(_{50})/(\mu)M</th>
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<tbody>
<tr>
<td>1</td>
<td>Neu5Ac2en</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>(N_3)</td>
<td>406</td>
</tr>
<tr>
<td>3</td>
<td>2a</td>
<td>290</td>
</tr>
<tr>
<td>4</td>
<td>2b</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>5</td>
<td>2c</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>2d</td>
<td>549</td>
</tr>
<tr>
<td>7</td>
<td>2e</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>8</td>
<td>2f</td>
<td>343</td>
</tr>
<tr>
<td>9</td>
<td>2g</td>
<td>133</td>
</tr>
<tr>
<td>10</td>
<td>2h</td>
<td>17</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>20</td>
</tr>
</tbody>
</table>

In our assay, Neu5Ac2en (Entry 1) was found to have an IC\(_{50}\) of 67 \(\mu\)M, 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 \(\text{M KOH}\), was found to be a weak neuraminidase inhibitor, with an IC\(_{50}\) of 406 \(\mu\)M. 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 in-
formation 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 IC₅₀ 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 IC₅₀. These results are in agreement with previous studies done in our lab on the synthesis and biological evaluation of C-glycoside-type neuraminidase inhibitors. 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 IC₅₀ 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 11 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 by classical procedures and stored on molecular sieves (4 Å). Nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were recorded at room temperature, in CDCl₃, MeOD or D₂O (500 MHz). Chemical shifts (δ) are indicated in ppm and coupling constants (J) in Hz. ESIMS were recorded with a LC/MSD trap. HRMS were performed with 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(SO₄)₂(NH₄)₆MoO₂O₇·4H₂O/H₂SO₄]. Flash chromatography was performed using silica gel 230–400 mesh.

Methyl 5-[Acetamido-3,5-dideoxy-4,7,8,9-tetra-O-acetyl-2-azido-D-glycero-a-d-galacto-non-2-ulopyranoside (3a): Methyl 5-[acetamido-3,5-dideoxy-4,7,8,9-tetra-O-acetyl-2-chloro-D-glycero-b-D-galacto-non-2-ulopyranoside (10.0 mmol, 5.0 g), tetrabutylammonium hydrogen sulfate (10.0 mmol, 3.4 g) and sodium azide (50.0 mmol, 3.26 g) were dissolved in a mixture of CH₂Cl₂/MeOH (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. NaHCO₃, dried with sodium sulfate and filtered. Evaporation of the solvent under diminished pressure afforded compound 3 as a white solid in 86% yield (4.8 g). ¹H NMR (500 MHz, CDCl₃, 25 °C): δ = 5.4–5.3 (m, 3 H, H-4, H-7, H-8), 5.0 (dd, J = 11.6, J = 10.3, J = 4.8 Hz, 1 H, 8-H), 4.4 (dd, J = 11.6, J = 3.0 Hz, 1 H, 1-9-H), 4.2–4.1 (m, 2 H, 5–H, 9-H), 3.9 (s, 3 H, COOME), 2.6 (dd, J = 13.0, J = 4.8 Hz, 1 H, 3eq-H), 2.15, 2.12, 2.04, 2.03 (4s, 4 CH₂COO), 1.9 (s, 3 H, CH₃OH), 1.8–1.7 (m, 1 H, 3ax-H) ppm. ¹³C NMR (125 MHz, CDCl₃, 25 °C): δ = 170.8, 170.6, 170.2, 170.0, 169.9 (4 CH₂COO, C-1), 167.1 (CH₂CONH), 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 (COOCH₃), 36.5 (C-3), 23.1 (CH₂CONH), 21.0, 20.8, 20.73, 20.71 (4 CH₂COO) ppm. ESIMS m/z, calcd. for C₉H₁₉N₃O₇ [M + Na]⁺ 539.2, found 539.2.

Methyl 5-[Acetamido-3,5-dideoxy-2-azido-D-glycero-a-D-galacto-non-2-ulopyranoside (1): Compound 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). ¹H NMR (500 MHz, MeOD, 25 °C): δ = 3.9 (s, 3 H, COOME), 3.9–3.8 (m, 4 H, 4, 5, 6-H and 9-H), 3.7 (dd, J = 11.8, J = 5.5 Hz, 1 H, 9-H), 3.5 (m, 2 H, 7, 8-H), 2.6 (dd, J = 12.8, J = 4.3 Hz, 1 H, 3eq-H), 2.0 (s, 3 H, AcNH), 1.6 (dd, J = 12.8, J = 11.2 Hz, 1 H, 3ax-H) ppm. ¹³C NMR (125 MHz, MeOD, 25 °C): δ = 175.9 (CH₂CONH), 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 (COOCH₃), 41.1 (C-3), 23.6 (CH₂CONH) ppm. HRMS m/z, calcd. for C₂₀H₂₈N₄O₁₂ [M + Na]⁺ 737.1179, found 737.1181.

Typical Procedure for the Click Reaction: Unprotected azido sialic acid donor 1 (0.2 mmol, 70 mg), alkyne (0.22 mmol), CuSO₄ (0.02 mmol, 3.2 mg) and sodium ascorbate (0.1 mmol, 20 mg) were successively added to a reaction flask. A mixture of H₂O/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 CH₂Cl₂/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)-1H-1,2,3-triazole-1-yl)-D-glycero-a-D-galacto-non-2-ulopyranoside Acid (2a): ¹H NMR (500 MHz, D₂O, 25 °C): δ = 7.9 (s, 1 H, H-1'·H-3'), 3.9–3.7 (m, 7 H, 4, 5, 6, 7, 8, 9-H), 3.5–3.4 (m, 2 H, 2·X-H), 3.1 (dd, J = 13.0, J = 4.5 Hz, 1 H, 3eq-H), 2.8 (t, J = 6.5 Hz, 2 H, 2·X-H), 2.1 (t, J = 13.0 Hz, 1 H, 3ax-H), 1.9 (s, 3 H, AcNH) ppm. ¹³C NMR (125 MHz, D₂O, 25 °C): δ = 175.0 (C-1), 170.4 (CH₂CO), 144.9 (C-
1,2,3-triazol-1-yl]-D-glycero-a-D-galacto-non-2-ulopyranosidic Acid (2b): 1H NMR (500 MHz, D2O, 25 °C): δ = 8.1 (s, 1 H, H-1'), 3.9–3.8 (m, 3 H, 4, 6, 7, 9-H'), 3.8–3.7 (m, 2 H, 8, 9-H'), 3.1 (dd, J = 12.7, J = 3.9 Hz, 1 H, 3eq-H), 2.1 (dd, J = 12.7, J = 11.0 Hz, 1 H, 3ax-H), 1.9 (s, 3 H, AcNH) ppm. 13C NMR (125 MHz, D2O, 25 °C): δ = 143.2 (C-1'), 142.8 (C-2'), 85.7 (OAc), 67.9 (C-6), 71.7 (C-8), 68.8, 67.9 (C-4, C-7), 63.5 (C-3'), 52.4 (C-5'), 38.0 (C-3acetal), 23.3 (C-6acetal), 23.1 (C-6ald), 163.0 (CH3CONMe), 1.88 and 1.86 (2s, 6 H, 2 C3H5CONMe). HRMS mlc, calcd. for C25H42N6NaO12 [M + Na]+ 541.2834, found 541.2827.

5-Acetamido-3,5-dideoxy-2-(4-acytelyloxy)-1H-1,2,3-triazol-1-yl]-D-glycero-a-D-galacto-non-2-ulopyranosidic Acid (2c): 1H NMR (500 MHz, D2O, 25 °C): δ = 8.1 (s, 1 H, H-1'), 3.9–3.8 (m, 3 H, 4, 6, 7, 9-H'), 3.8–3.7 (m, 2 H, 8, 9-H'), 3.1 (dd, J = 12.7, J = 3.9 Hz, 1 H, 3eq-H), 2.1 (dd, J = 12.7, J = 11.0 Hz, 1 H, 3ax-H), 1.9 (s, 3 H, AcNH) ppm. 13C NMR (125 MHz, D2O, 25 °C): δ = 143.8 (C-1'), 142.3 (C-2'), 85.4 (OAc), 67.9 (C-6), 71.7 (C-8), 68.8, 67.9 (C-4, C-7), 63.5 (C-3'), 52.4 (C-5'), 38.0 (C-3acetal), 23.3 (C-6acetal), 23.1 (C-6ald), 163.0 (CH3CONMe), 1.88 and 1.86 (2s, 6 H, 2 C3H5CONMe). HRMS mlc, calcd. for C25H42N6NaO12 [M + Na]+ 541.2834, found 541.2827.

5-Acetamido-3,5-dideoxy-2-(4-acytelyloxy)-1H-1,2,3-triazol-1-yl]-D-glycero-a-D-galacto-non-2-ulopyranosidic Acid (2d): 1H NMR (500 MHz, D2O, 25 °C): δ = 8.1 (s, 1 H, H-1'), 3.9–3.8 (m, 3 H, 4, 6, 7, 9-H'), 3.8–3.7 (m, 2 H, 8, 9-H'), 3.1 (dd, J = 12.7, J = 3.9 Hz, 1 H, 3eq-H), 2.1 (dd, J = 12.7, J = 11.0 Hz, 1 H, 3ax-H), 1.9 (s, 3 H, AcNH) ppm. 13C NMR (125 MHz, D2O, 25 °C): δ = 143.2 (C-1'), 142.8 (C-2'), 85.7 (OAc), 67.9 (C-6), 71.7 (C-8), 68.8, 67.9 (C-4, C-7), 63.5 (C-3'), 52.4 (C-5'), 38.0 (C-3acetal), 23.3 (C-6acetal), 23.1 (C-6ald), 163.0 (CH3CONMe), 1.88 and 1.86 (2s, 6 H, 2 C3H5CONMe). HRMS mlc, calcd. for C25H42N6NaO12 [M + Na]+ 541.2834, found 541.2827.
Methyl 5-acetamido-9-O-(p-methoxybenzyl)-3,5-dideoxy-4,7-di-O-phenyl-2-thio-d-glycero-d-a-galacto-non-2-ulopyranoside (7): 1H NMR (500 MHz, MeOD, 25 °C): δ = 7.5–7.7 (m, 7 H, 5x H₂PhPMB), 6.9 (d, J = 8.6 Hz, 2 H, 2x H₂PMB), 5.9 (d, J = 8.3 Hz, 1 H, 6-H), 5.1 (dt, J₂ = 9.2 Hz, J₁ = 2.6 Hz, 1 H, 1-H), 4.9 (dd, J₁ = 11.6 Hz, J₂ = 4.7 Hz, 1 H, 7-H), 4.5–4.4 (m, 2 H, OCH₂Ph), 4.1–4.0 (m, 1 H, 5-H), 3.9–3.8 (m, 2 H, 8-H, 9-H), 3.8 (s, 3 H, OMe), 2.8 (dd, J₁ = 12.8 Hz, J₂ = 4.7 Hz, 1 H, 3eq-H), 2.1, 2.0 (2 H, 6 OAc), 2.1–2.0 (m, 1 H, 3ax-H), 1.9 (s, 3 H, AcNH) ppm. 13C NMR (125 MHz, MeOD, 25 °C): δ = 170.6, 170.5, 170.3 (COOME, 2 COOMe), 168.0 (CH₂CONH), 159.2 (C-5’), 136.2 (2 CH₂PMB), 136.0 (C-2’), 129.9 (4 α CH₂PMB), 129.6 (2 C-3’), 129.0 (1 CH₂PMB), 128.8 (2 CH₃PMB), 113.7 (2 C-4’), 87.5–8.2 (7, 96-C), 74.6 (C-4, C-7), 73.4 (C-6), 72.9 (C-3), 70.5 (OCH₂Ph), 68.7 (C-4, C-7), 67.7 (C-9), 56.7 (MeOCH₃), 55.3 (C-5’), 52.5 (2 C-5), 52.5 (2 C-5), 49.5 (1, 9 H, 6-H), 45.3 (J = 11.0 Hz, 5-H), 43.4 (4, 2 H, 2x H₂PMB), 42.1–41.8 (m, 2 H, 2x H₂PMB), 41.4 (2 H, 2x H₂PMB), 37.9 (CH₂OAc), 37.9 (C-3), 23.0 (MeOCONH), 21.1 and 20.9 (2 MeOCONH) ppm. HMRs m/z, calcd. for C₃₅H₃₉N₅O₇S [M + Na]⁺ 680.2136, found 680.2138.

Propargyl [Phenyl 5-acetamido-3,5-dideoxy-8-O-propargyl-2-thio-d-glycero-d-a-galacto-non-2-ulopyranoside (8): Compound 7 (0.3 mmol, 200 mg), barium oxide (0.3 mmol, 200 mg), 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 h. The reaction was quenched with pTosOH and filtered through a pad of Celite. The solids were washed with MeCN, and the filtrates were combined and the solvents evaporated. The residue was purified by flash column chromatography (ethyl acetate/methanol, 1:0 to 1:1), to afford 8 as a white solid in 36% yield (7 mg). 13C NMR (125 MHz, DMSO-d₆, 25 °C): δ = 170.6 (COOME, 2 COOMe), 168.0 (CH₂CONH), 159.2 (C-5’), 136.2 (2 CH₂PMB), 136.0 (C-2’), 129.9 (4 α CH₂PMB), 129.6 (2 C-3’), 129.0 (1 CH₂PMB), 128.8 (2 CH₃PMB), 113.7 (2 C-4’), 87.5–8.2 (7, 96-C), 74.6 (C-4, C-7), 73.4 (C-6), 72.9 (C-3), 70.5 (OCH₂Ph), 68.7 (C-4, C-7), 67.7 (C-9), 56.7 (MeOCH₃), 55.3 (C-5’), 52.5 (2 C-5), 52.5 (2 C-5), 49.5 (1, 9 H, 6-H), 45.3 (J = 11.0 Hz, 5-H), 43.4 (4, 2 H, 2x H₂PMB), 42.1–41.8 (m, 2 H, 2x H₂PMB), 41.4 (2 H, 2x H₂PMB), 37.9 (CH₂OAc), 37.9 (C-3), 23.0 (MeOCONH), 21.1 and 20.9 (2 MeOCONH) ppm. HMRs m/z, calcd. for C₃₅H₃₉N₅O₇S [M + Na]⁺ 680.2136, found 680.2138.
8 propargyl acceptor 10 (11 μmol, 5 mg), CuSO4 (1.2 μmol, 0.2 mg) and sodium ascorbate (6 μmol, 1.2 mg). A mixture of H2O/ButOH/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 CH2Cl2/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). 1H NMR (500 MHz, D2O, 25°C): δ = 8.1 (s, 1 H, 1'-H), 7.5 (d, 2 H, J = 7.5 Hz, 2Hsps), 7.4 (d, J = 7.5 Hz, 1 H, 1''-H), 4.6 (d, J = 8.5 Hz, 1 H, 1 3''-H), 3.9–3.4 (m, 14 H, 2 × 4,5,6,7,8-H, 4 × 9-H), 3.2 (dd, J = 12.5, J = 4.0 Hz, 1 H, 3eq-H), 2.9 (dd, J = 12.9, J = 4.5 Hz, 1 H, 3ax-H), 2.1 (m, J = 12.5, J = 11.5 Hz, 1 H, 3ax-H), 1.9, 1.8 (2s, 6 H, 2 AcNH), 1.9–1.8 (m, 1 H, 3ax-H) ppm. 13C NMR (125 MHz, D2O, 25°C): δ = 174.3 (4 C-1), 173.1, 172.6, 172.5, 172.3 (16 C-2), 75.9, 75.0, 74.2, 71.9, 70.0, 68.1, 66.8 (2 C-4, 2 C-6, 2 C-7, 2 C-8), 63.2, 62.6 (2 C-9), 61.3 (C-3 m), 51.5, 49.8 (C-2, C-5), 38.9, 37.5 (2 C-3), 22.1 (2 CH3CONH) ppm. HRMS m/z, calcd. for C31H42N5O16S [M – H]– 772.2353, found 772.2346.

Amino-4-Methylumbelliferyl-N-acetyl neuraminic acid (MUNANA), N-Acetyl-2,3-dehydro-2-deoxy neuraminic 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 – 1 μM in 0.1 molar sodium acetate buffer, pH 5.0 in triplicate, to determine IC50 of each inhibitor. Neuraminidase (0.5 μM) 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 μM) was added and the reaction mixture was incubated for 1 h 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 IC50 using software from GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

Supporting Information (see also the footnote on the first page of this article): Spectral data, 1H, 13C NMR of compounds 1, 2a–h, 3, 6–14 and IC50 curves of 2a–h, 11, 14, Neu5Ac2en.

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