Design and Synthesis of \textit{N}-Acyl Modified Trivalent Sialic Acid Inhibitors of Adenovirus type 37

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Master's Level

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"Life battles do not always go to the stronger or faster man. But sooner or later the man who wins, is the man who thinks he can." (Vince Lombard). As it is written "…It is not by strength that one prevails" (1 Samuel 2:9)
Dedication

I dedicate this thesis to my God Jesus Christ, and to the pillars of my life: my parents, my siblings, and my everlasting love.

I might not know where the life’s road will take me, but walking with You, my God, through this journey has given me strength, success and every meaning for my life. Without you, I am nothing!

Mama and Papa, I could never thank you enough for all of the love, the support and the encouragement. For the rest of my life, I am indebted to you for all of the values that you have instilled in me. You have given me faith because you believe.

Marko, Nana and Marina, thank you so much for all that you've done for me! I can't fully express how much I love you and appreciate you.

Mariam, you are the greatest gift of my life. You came into my life to give it a whole new meaning. I love you more than you imagine!
Abstract

Epidemic keratoconjunctivitis (EKC) is one of the threats to the public health that are associated with adenoviruses of serotype 8 (Ad8), Ad19 and Ad37. These serotypes bind to and infect the human corneal epithelial (HCE) cells to cause severe and highly contagious ocular disease that may rise up to an outbreak. This viral infection is promoted by the attachment of the virion to the cellular receptors through the viral fiber proteins which are protruding from the virus particles. Ad37 fibers carry a terminal homotrimer domain (knob) that utilizes sialic acids-containing glycolconjugates as cellular receptors to attach the host cells and promote infection. Recently, trivalent sialic acid-conjugates have been verified to bind to the Ad37 fiber knob efficiently to inhibit Ad37 cell attachment and infection of HCE cells. As a result, a potent trivalent sialic acid inhibitor of Ad37 was reported. Herein, we present design and synthesis of N-acyl modified trivalent sialic acid derivatives of the most potent inhibitors of EKC-causing Ad37. According to the extensive studies of Ad37 structural features we aimed to improve the inhibitory potency of this most potent inhibitor.

Keywords

Adenoviruses, Ad37, Carbohydrates, Carbohydrate-protein interaction, Click chemistry, Copper catalyzed azide-alkyn cycloaddition (CuAAC), Epidemic keratoconjunctivitis (EKC), Glycoconjugates, Glycosylation, Sialic acid, Trivalent, Triazole, X-ray crystal structure.
# Table of Contents

1. Introduction ...................................................................................................................... 1
   1.1 Antiviral drug development ....................................................................................... 1
   1.2 Adenovirus ............................................................................................................... 1
      1.2.1 Treatment of Adenoviruses ............................................................................... 1
      1.2.2 Adenovirus structure ......................................................................................... 1
      1.2.3 Mechanism of interaction .................................................................................. 1
      1.2.4 Lead identification .............................................................................................. 2
   1.3 Carbohydrates in drug discovery ................................................................................. 2
   1.4 Sialic acids ................................................................................................................. 3
      1.4.1 Structures and functions of sialic acids ............................................................... 3
      1.4.2 Determination of sialic acid anomericity ............................................................. 3
      1.4.3 Mechanistic aspects of glycosylation reactions ................................................... 3
2. Aim of the thesis ................................................................................................................. 4
3. Results and discussion ....................................................................................................... 5
   3.1 Structure-based design ............................................................................................... 5
   3.2 Synthesis ..................................................................................................................... 6
      3.2.1 General synthetic strategy (Scheme 3.1) ............................................................. 6
      3.2.2 Synthesis of N-propanoylamide 2-thiophenyl α-sialyl glycoside (Scheme 3.2) ... 6
      3.2.3 Synthesis of N-propanoylamide 2-O-propyl-(3-azido)-α-sialyl glycoside (Scheme 3.3) ........................................................................................................... 6
      3.2.4 Synthesis of trivalent sialic acid via "click" reaction (Scheme 3.4) ......................... 8
4. Conclusion .......................................................................................................................... 8
5. Experimental section ......................................................................................................... 9
   5.1 General chemistry ........................................................................................................ 9
   5.2 Chemical synthesis .................................................................................................... 9
6. Acknowledgement ............................................................................................................. 14
7. Reference .......................................................................................................................... 14
### List of abbreviations

<table>
<thead>
<tr>
<th>Ac</th>
<th>Acetyl</th>
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<tbody>
<tr>
<td>Ad</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butyloxycarbonyl</td>
</tr>
<tr>
<td>CAR</td>
<td>Coxsackie/adenovirus receptor</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Copper-catalyzed azide–alkyne cycloaddition</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-Diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EKC</td>
<td>Epidemic keratoconjunctivitis</td>
</tr>
<tr>
<td>eq</td>
<td>Equivalent</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>HA</td>
<td>Haemagglutinin</td>
</tr>
<tr>
<td>HAdV</td>
<td>Human adenovirus</td>
</tr>
<tr>
<td>HATU</td>
<td>O-(7-azabenzotriazole-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HCE</td>
<td>Human corneal epithelial</td>
</tr>
<tr>
<td>HSA</td>
<td>Human serum albumin</td>
</tr>
<tr>
<td>IC\textsubscript{50}</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>Kdn</td>
<td>Ketodeoxy nonulosonic acid</td>
</tr>
<tr>
<td>LCMS</td>
<td>Liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometry</td>
</tr>
<tr>
<td>NA</td>
<td>Neuraminidase</td>
</tr>
<tr>
<td>Neu5Ac</td>
<td>Neuraminic acid</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>Pro</td>
<td>Proline</td>
</tr>
<tr>
<td>R\textsubscript{f}</td>
<td>Retention factor</td>
</tr>
<tr>
<td>TBAI</td>
<td>tetra-N-butylammonium iodide</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TfO</td>
<td>Trifluoromethanesulfonate, triflate</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
<tr>
<td>Tol</td>
<td>Toluene</td>
</tr>
<tr>
<td>Tyr</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>-----</td>
<td>----------</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
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1. Introduction

1.1 Antiviral drug development

Over the past three decades, a rapid increase in the demand for antiviral medications has been reflected by moving from three to more than forty marketed drugs.1 Nevertheless, the market is still lacking efficient treatments for several viral infections.

Antiviral drug design is generally based on targeting the viral proteins which are vital for the viral life cycle.2 Drugs that block the viral enzyme are thus indirectly inhibiting the synthesis of viral components and, consequently, the infection promotion.3 Although this strategy is used for a long time, it remains limited to focus only on targeting the processing enzymes.

Since the beginning of this century, studies on the life cycle of viruses have provided new strategies for antiviral drug design. For instance, some marketed antiviral agents target the virus attachment to the host cell while others inhibit the virus cell-fusion process.5

1.2 Adenovirus

Human Adenoviruses (HAdV) have been identified and first isolated from adenoidal tissue of children after tonsillectomy in 1953 by P. Rowe et al.4 thence the name adenovirus. Since then, adenoviruses have been extensively characterized and seven species (A to G) including 54 serotypes of adenovirus have been identified.5,6 The adenovirus family (Adenoviridae) can infect various cells and causes a wide variety of infections such as upper and lower respiratory tract infections, conjunctivitis, gastroenteritis and cystitis, where a specific serotype is responsible for each infection.7,8 Among those, epidemic keratoconjunctivitis (EKC) is one of the serious threats to the public health in consideration to ocular infection that is associated with serotypes Ad8, Ad19 and Ad37. EKC-causing serotypes specifically infect the human corneal epithelial (HCE) cells to cause severe and contagious disease that may rise up to outbreaks as reported worldwide (Figure 1.1).9,10 Recently, EKC outbreaks were reported in the northern part of Germany.11

1.2.1 Treatment of Adenoviruses

Currently, there is no effective treatment available for adenoviral infections in general and for EKC in particular. Fortunately, our body can afford natural treatment for EKC by alleviating the symptoms naturally within 1–3 weeks.12 Nonetheless, the patients are suffering from severe symptoms during this period.11 Importantly, the patients can still transmit the disease to others by bodily fluids and this even before the symptoms arise, which can lead to adenoviral outbreaks.11,12

![Figure 1.1 An eye with severe epidemic keratoconjunctivitis infection. Picture has been used with permission granted from the editor Mark B. Abelso.12](image)

Therefore, effective treatment for such infection is highly demanded. Several attempts have been made to find a treatment for this disease. Unfortunately, none of these attempts led to an effective medication. For instance, steroids were tested because of their anti-inflammatory effects; while the clinical trials have shown weak effect, most observed is the adverse side effect of prolongation of the disease.13,14 Also, various virustatic agents have been evaluated both in vitro and in vivo but clinical trials have shown mild or even no effect.11

1.2.2 Adenovirus structure

Detailed three-dimensional structure of adenovirus has been determined based on combination of X-ray crystallography and cryo-electron microscopy by Stewart, P. L. et al.15 The adenovirus particle (virion) is a non-enveloped icosahedral capsid, 70 – 100 nm in diameter that contains linear, double strand-DNA genome (Figure 1.2). This capsid consists of 252 capsomeres, where the 240 capsomeres forming the faces and the edges are called hexon and the 12 capsomeres forming vertices are called penton.16 The names hexon and penton respectively refer to the hexagonal and pentagonal shapes of the protein subunits of each capsomere. Each of the penton bases has a fiber protruding from the capsid surface and terminated with a globular domain termed "knob". This fiber knob gives a characteristic morphology for the adenovirus particles generally,17 but for Ad37 particles particularly,18 as discussed in the next section.

1.2.3 Mechanism of interaction

Human adenovirus life cycle includes several steps (Figure 1.3) in which the attachment of the virion to the cellular receptors is the trigger that promotes infection.16 In the 1990s’ it has been confirmed that adenovirus uses its fiber knob domain to interact with its cellular receptors,19 and most of the human adenoviruses, but
not all, bind to coxsackie adenovirus receptor (CAR). It has also been verified that the fiber knobs characterize the intercellular interactions of different adenoviruses serotypes.

For Ad37, the fiber knob consists of homotrimer proteins (Figure 1.2), wherein each subunit presents a separate binding site. This homotrimer fiber knob was demonstrated to bind to sialoglycoprotein receptors. Thus, structures that mimic sialoglycoprotein cellular receptors can effectively bind to the virion and hinder its attachment to the host cells to inhibit the infections accordingly.

Recently, glycan array screening and structural studies of the trimeric Ad37 knob domain have revealed that the knob specifically binds to a branched hexasaccharide in the GD1a ganglioside. Gangliosides are oligoglycosphingolipids that are located on the cell-surface and contain one or two sialic acid residues that participate in intermolecular interactions and cell recognition. Also, it has been demonstrated that these two sialic acid residues of GD1a ganglioside dock into two out of three sialic acid-binding sites that are presented by the fiber knob proteins.

1.2.4 Lead identification

On the basis of Ad37 fiber knob structure features, and previous results, a trivalent sialic acid-conjugate (ME0322) (Figure 1.4) was reported as a potent inhibitor of Ad37. Its potency was proven to be at least 1000 times greater than sialic acid. This trivalent sialic acid-conjugate was specifically designed to fit into the binding sites of knob proteins which are separated by a distance of ca 10 Å. Therefore, a flexible linker and a compact scaffold were selected for conjugation with three units of sialic acid as depicted in Figure 1.4.

The virus-binding assay has shown ME0322 to have an IC50 value of ca 14 μM, while the infection assay has shown its inhibitory potency to be four orders of magnitude more potent than GD1a hexasaccharide.

1.3 Carbohydrates in drug discovery

Carbohydrates and their glycoconjugates play crucial roles in various biological processes such as in cell-cell recognition, cellular-immune response, signal transduction, bacterial adhesion, viral infections, and tumor progression. Exploitation of such interactions assist the drug discovery process, such as the case of sialic acid in EKC-causing adenoviruses as discussed earlier. Nevertheless, the high polarity of carbohydrates reflects their poor pharmacokinetic properties which limit their ability to work perfectly as drug-like compounds.

Figure 1.2 Right: Schematic 3D structure of adenovirus icosahedral capsid. Left: Cross-sectional view of the viral capsid. The picture has been used with permission granted from ViralZone: www.expasy.org/viralzone, Swiss Institute of Bioinformatics.

Figure 1.3 Adenoviral replication cycle. Virion first attaches to the cellular receptors (which is CAR in this case), followed by internalization to the host cell, and further steps leading to synthesis new virions that finally released by cell lysis.

Figure 1.4 Structure of ME0322 (Trivalent sialic acid-conjugate).

* See section 1.4 for sialic acid structures
That is also a reason for the presence of relatively few carbohydrate-based drugs in the market nowadays. Therefore, one might think that the development of antiviral drug from carbohydrate-based compound (sialic acid-conjugates) is not a good strategy. Having said that, in this particular case of EKC-causing Ad37 infection, the antiviral agent is directly targeting the eye (HCE) and it can be administrated topically via cream, eye drops or ointment, thereby such drawback can be avoided.

1.4 Sialic acids

1.4.1 Structures and functions of sialic acids

Sialic acid is a 9-carbon, acidic α-keto sugar that is present in mammalian and avian tissues in the form of lipooligosaccharides and glycoproteins. It is the major glycan component that is released by mild hydrolysis of brain glycolipids or salivary mucins. There are two common sialic acids structures (Figure 1.7). The difference between them is the substitution at C-5, as highlighted in Figure 1.7, which could be N-acetyl group giving Neuraminic acid (Neu5Ac) or a hydroxyl group to give ketodeoxy nonulosonic acid (Kdn). All other sialic acids are metabolically derived from these two.

Sialic acids exist in macromolecules in a variety of glycosidic linkages, most common is α(2-3) and α(2-6) to galactose or lactose, but also as α(2-8) and α(2-9) linkage in polysialic acid.

\[ \text{Figure 1.7 Structures of two common sialic acids. A) 5-acetamido-2-keto-3,5-dideoxy-D-glycero-D-galactonononic acid (N-acetylneuraminic acid; Neu5Ac). B) 2-keto-3-deoxy-D-glycero-D-galactonononic acid (2-keto-3-deoxynononic acid, Kdn).} \]

Sialic acids play major roles in various biological processes. Interestingly, many pathological microbes exploit sialic acids residues to promote infection of the host cells. For example, influenza virus particle utilizes the viral enzyme (neuraminidase (NA)) to cleave the sialic acid residue from the cell glycoprotein to allow the virus to reach the cell surface. Once that happens, the virion makes use of another viral protein (haemagglutinin (HA)) to recognize and bind to sialic acid followed by adsorption and penetration into another host cell.

1.4.2 Determination of sialic acid anomericity

The anomeric configuration of the glycoconjugate linkages are often determined by NMR spectroscopy. Sialic acid anomericity can be elucidated by the variation of the chemical shifts of H-3eq, H-3ax and H-4 between α-anomer and β-anomer. In addition, heteronuclear multiple-bond correlation (HMBC) experiment is used to confirm the α-anomeric configuration by measuring the coupling constant (J) between C-1 and H-3ax to be ca 6.7 Hz.

\[ \text{Figure 1.8 Structures of α- and β-anomeric configurations of sialic acid A and B respectively. The arrows show the positions of the -COOH group (C-1) in relation to H-3ax for each anomer.} \]

1.4.3 Mechanistic aspects of glycosylation reactions

Glycoside synthesis is a very important reaction that yield oligosaccharides or glycoconjugate compounds such as glycoproteins, glycolipids and many other carbohydrate-based natural products. Various glycosylation methods have been developed to date. In principle, they are divided into two steps; first is the activation of the anomeric center by the aid of a promoter or catalyst to release the glycosyl donor, and second is the glycosylation reaction, in which the activated glycosyl donor is captured by the glycosyl acceptor to form the glycoside bond. Controlling the regio- and stereoselectivity of such reaction is crucial to obtain the product of interest. Scheme 1.1 summarizes general aspects of different glycosylation methods.

Synthesis of α-sialyl glycoside 4 (Scheme 1.2) is one of the challenging glycosylation reaction in essence of stereoselectivity with satisfactory yield. That is not only because of the sterically hindered anomeric center at C-2, but also due to the presence of the carboxylic acid group which lead to the electronically disfavored oxocarbenium intermediates 2 and 3. Moreover, the absence of substituent at C-3 precludes neighboring group participation that can be used to control the stereoselectivity of the outcome. Most challenging is the competitive elimination by losing H-3, due to those above reasons, which leads to 2,3-dehydro derivative
Scheme 1.1 Overview of the glycosylation methods under basic or acidic catalyzed activation, and different promoters.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fisher - Hellerich: acid catalyzed activation</td>
</tr>
<tr>
<td>B</td>
<td>Koenigs - Knorr: X= Cl,Br; F-Activation X= F-Activation X= S-Activation</td>
</tr>
<tr>
<td>C</td>
<td>Anomeric O-Alkylation: base activation</td>
</tr>
<tr>
<td>D</td>
<td>Trichloroacetimidate Activation: PO(OH)2, PO(OH)3 - Activation SO2(OH)-, SO2(OH)2 - Activation</td>
</tr>
</tbody>
</table>

Glycosylation: "X-philic" Promoter e.g. Heavy metal salts: Ag+, Hg+
Glycosylation: Mild Acid Catalyst e.g. BF3, Et2O, TMSOTf

Scheme 1.2 Glycosylation of sialic acid donor with competing elimination product (glycal).

OP = protected hydroxyl group

Also, one has to bear in mind that the thermodynamically favored product is the β-glycoside not the α-anomer, and that is because of the anomeric effect. Therefore, many state-of-the-art strategies have been developed to overcome and control such problems and to obtain the desired stereoselectivity in satisfactory yields.37

2. Aim of the thesis

This thesis challenges the synthesis of sialic acid-based potential antiviral agents that inhibit EKC-causing Ad37 binding to and infection of the HCE cells. These inhibitors are mimicking the trivalent sialic acid-conjugate that has been reported earlier as a potent inhibitor (ME0322)29 and also derivatives of the most
potent inhibitors (ME0385 and ME0386) that have been developed recently (Figure 3.2) (unpublished results).

According to previous studies on the Ad37 knob domain, we aimed to improve the potency of ME0385 and ME0386 by adding additional hydrophobic interaction between those ligands and the target proteins. In order to achieve that, the N-acyl group which is oriented toward a hydrophobic pocket was planned to be modified to more lipophilic acyl group. Thus, a strategy to synthesis the N-acyl modified sialic acid was developed and followed by synthesis of two trivalent N-acyl modified sialic acid-conjugates with two different linker lengths.

3. Results and discussion

3.1 Structure-based design

In a previous study, the crystallographic analysis of ME0322 (Figure 1.4) in complex with the trimeric Ad37 knob domain did unfortunately not provide any structural information about the linker nor the core scaffold (Figure 3.1).28 Thus, solely the terminal sialic acid residues were resolved leaving the existence of interactions between the protein and the linker unclear. A tentative hypothesis would be that no defined contacts between the core scaffold of ME0322 and the target proteins exist.

Then our group synthesized trivalent sialic acid derivatives with a shorter and more rigid linker which led to two new inhibitors ME0385 and ME0386 (Figure 3.2). These new inhibitors were evaluated and demonstrated improved inhibitory effect (IC$_{50}$ < 5.0 µM) compared to ME0322 (unpublished results). The crystallographic data from this later study provided more information about the interactions within the binding sites, and the structures of the inhibitors in complex with the knob domain were fully resolved. Interestingly, although the inhibitory potency has been improved the linkers do not have any additional interactions with the proteins.

Figure 3.1 Crystal structure of Ad37 fiber knob protein in complex with ME0322. Figure is used with permission granted from the publisher of Angewandte Chemie: John Wiley and Sons.

Figure 3.2 Structures of the developed trivalent sialic acid-conjugates inhibitors of Ad37.

Extensive structural studies on Ad37 fiber knob domain (Figure 3.3 and 3.4) have shown the possibility to improve the binding potency of such inhibitors further. Indeed, the N-acyl group being oriented toward a hydrophobic pocket; increasing the lipophilicity of this N-acyl moiety may create additional van der Waals interactions to increase the binding affinity and the potency as a result. However, several attempts have been done previously to increase the binding affinity of the sialic acid-HSA conjugate by making use of this hydrophobic pocket. Unfortunately, none of these attempts succeed to find an inhibitor as potent as the original sialic acid-HSA conjugate.28 That could be due to the binding site conformation is rigid enough and does not allow a larger substituent to fit and interact perfectly within the hydrophobic pocket. Thus, the variation of N-acyl group for this sialic acid-HSA conjugate did not show any promising results.28 Also, the relatively big multivalent sialic acid-HSA conjugates (74381 g/mol) and its longer linker could play important roles in this interaction.

Figure 3.3 Sialic acid-knob domain of the Ad37 fiber protein interaction. The molecular surface of the fiber knob is colored blue for polar surface, brown for nonpolar parts, and green for intermediate polarity. Four main interactions have been highlighted with the amino acids residues.38 one salt bridge

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>R</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME0385</td>
<td>H</td>
<td>1</td>
</tr>
<tr>
<td>ME0386</td>
<td>H</td>
<td>2</td>
</tr>
<tr>
<td>ME0407</td>
<td>CH$_3$</td>
<td>2</td>
</tr>
<tr>
<td>ME0408</td>
<td>CH$_3$</td>
<td>1</td>
</tr>
</tbody>
</table>
between carboxylic acid (COO—Lys345), two hydrogen bonds (4-OH-Tyr312, 5-NH-Pro317), and one hydrophobic interaction between the N-acetyl group of the sialic acid. The figure is used with permission granted from the publisher of Journal of Medicinal Chemistry: American Chemical Society. 

Therefore, one may think that the N-acyl modified sialic acid of shorter and more compact linker (ME0385 and ME0386) still can reveal significant results and provide structural information that may be used to investigate/conclude this hydrophobic interaction with Ad37 target protein. For this reason, we planned to make a modification on sialic acid moiety at C-5 where the N-acetyl group was replaced with N-propanoyl group to obtain ME0407 and ME0408 which are corresponding to ME0386 and ME0385 respectively (Figure 3.2).

![Figure 3.4](image-url) The four main interactions within the binding site with sialic acid residue as described in Figure 1.4, and the hydrophobic pocket formed by Tyr312/C, Tyr 308/B of another monomer, Pro317/B, and Val322/C. Where A, B, C are the subunits (monomer) of the homotrimer. Figure is used with permission granted from the publisher of Nature Medicine: Nature Publishing Group.

### 3.2 Synthesis

#### 3.2.1 General synthetic strategy (Scheme 3.1)

Herein, we report an efficient general synthetic strategy not only for the synthesis of our main targets (ME0407 and ME0408), but also for divergent synthesis of various derivatives as outlined in scheme 3.1. By retrosynthetic analysis our target molecules can be disassembled to start from the commercially available sialic acid. Starting from the α-anomeric form of sialic acid, the more stable protected thiosialoside donor I was prepared that can be stored for long time, and survive a range of reaction conditions associated with our strategy. This thiosialoside donor is used as intermediate for divergent synthesis to reach ME0385 and/or ME0386 via glycosylation. Alternatively, it is also used to prepare the corresponding ammonium salt as a very important intermediate II that afford the precursors to make a library of the N-acyl modified sialic acids. Each of these N-acyl modified thiosialosides can be considered as another intermediate III to be followed by synthesis of series of derivatives for the most potent inhibitors (ME0385 and ME0386). In one case, the N-propanoylamide thiosialoside donor IV was connected to different linkers via glycosylation reaction to obtain our main targets (ME0407 and ME0408).

#### 3.2.2 Synthesis of N-propanoylamide 2-thiophenyl α-sialyl glycoside (Scheme 3.2)

The commercially available sialic acid (N-acetylsialaminic acid) 6 was used to prepare the protected phenyl thiosialoside 9 with three-step one pot reaction. Firstly, methyl ester of sialic acid 7 was prepared, followed by acetylation and preparation of chloride-β-sialyl glycoside 8a. Treatment of the glycosyl chloride 8a with thiophenol in the presence of Hüning’s base yielded the α-anomer of thiosialoside 9a as the major outcome beside β-anomer and elimination product (glycal) 8b in 7:1:2 ratio respectively. The acetamide group was then Boc-protected to form N,N-Ac,Boc thiophenyl sialyl glycoside 10a. Pure α-anomer was obtained after Zemplén deacetylation to give N-Boc thiophenyl α-sialyl glycoside 11 in 58% yield over four steps. Then, O-acetylation was performed to get the corresponding peracetylated product 12. Cleaving the Boc protection with TFA (aq.) in CH₂Cl₂ afforded the ammonium salt 13. Finally, amide coupling was performed using propanoic acid in the presence of HATU as a coupling agent to afford the corresponding N-propanoylamide α-sialyl glycoside 14 in combination with tetramethyl urea in 8:1 ratio and 85% yield.

#### 3.2.3 Synthesis of N-propanoylamide 2-O-propyl-(3-azido)-α-sialyl glycoside (Scheme 3.3)

The N-propanoylamide α-sialyl glycoside 14 was used as a glycosyl donor for glycosylation reaction, while two different linkers (2-bromo ethanol 15 and 3-bromo propan-1-ol 16) were used as glycosyl acceptors. Glycosylation reaction was performed by making use of interhalogen/silver trifluoromethanesulfonate (IBr/AgOTf) promoted glycosylation. The reaction was kinetically controlled at -73 °C with MeCN/CH₂Cl₂ (3:2) as solvent. Therefore, the stereoselectivity of the outcome was controlled to afford 17 and 18 in 7:1 and 6:1 α- to β-anomeric ratio and 95-98% yield respectively. Then the bromo group was substituted by azido group with sodium azide and TBAI as a catalyst to obtain the corresponding azido derivatives 19 and 20 in quantitative yield. Ultimately, Zemplén deacetylation followed by chromatographic separation afforded the α-anomers 21 and 22 in 45-55% yield over three steps.
Scheme 3.1 Outline of the general synthetic strategy for the synthesis of diverse trivalent sialic acid-conjugates.

Scheme 3.2 Synthesis of N-acyl modified sialic acid.

Reagents and conditions: (a) MeOH, Dowex 50W8, rt, 4 h. (b) AcCl, MeOH/AcOH 3:1, 0 °C → room temperature, 48 h. (c) PhSH, DIPEA, CH₂Cl₂, room temperature, dark, overnight. (d) Boc₂O, DMAP, THF, refluxed 3 h. (e) (1) NaOMe, 0.03 M MeOH, room temperature, 4 h. (2) HOAc conc. drops (f) Ac₂O, Pyridine, room temperature, overnight. (g) 90% TFA/CH₂Cl₂ 2:1, room temperature, 2 h. (h) CH₃CH₂COOH, HATU, DIPEA, CH₂Cl₂, room temperature, 21 h.
Scheme 3.3 Synthesis of N-acyl modified sialic acid

Reagents and conditions: (a) (1) 15 or 16, activated molecular sieve 3Å, AgOTf, IBr, MeCN/CH₂Cl₂, -73°C, 5 h (2) DIPEA, room temperature, 1 h (b) NaN₃, TBAI, DMSO, room temperature, 6 h (c) NaOMe, 0.03 M MeOH, room temperature, 2-3 h.

Scheme 3.4 Synthesis of Trivalent N-acyl modified sialic acid via "click" reaction.

Reagents and conditions: (a) Tripropargylamine, CuSO₄, Sodium ascorbate, THF/H₂O, room temperature, overnight (b) LiOH, MeOH, room temperature, 12 h.

3.2.4 Synthesis of trivalent sialic acid via "click" reaction (Scheme 3.4)

Trivalent sialic acid was synthesized by utilizing copper catalyzed azide-alkyne cycloaddition (CuAAC) which so-called "click reaction". The deacetylated α-sialyl glycoside azido derivatives 21 and 22 were reacted with tripropargylamine in the presence of copper (II) salt and ascorbate salt to yield the trivalent sialic acid-conjugates 23 and 24 in 35-50% yield. Finally, saponification of methyl ester was performed with LiOH to get the corresponding acid 25 and 26 (ME0407 and ME0408) as the targets of interest in 75 and 72% yields respectively.

4. Conclusion

This study presents a successful design and synthesis of trivalent N-propanoylamide sialic acid-conjugates as potential antiviral agents for EKC-causing Ad37. These trivalent ligands are constituted of three sialic acid residues that are connected together via compact and rigid linkers.

During this synthesis, advanced and robust chemical techniques have been applied. Glycosylation reaction of sialic acid was performed utilizing interhalogen/silver triflate activation of the thiosialoside with satisfactory yields and stereoselectivities. Also, the yield of the amide coupling reaction was enhanced in comparison with the literature.

Moreover, chemical transformation from monovalent to multivalent (trivalent) was performed by making use of CuAAC. This later transformation was achieved with high regioselectivity, thus, exclusively affording 1,4-disubstituted regioisomer.
As a result, two new potential antiviral agents (ME0407 and ME0408) for EKC-causing Ad37 have been synthesized with different linker lengths aiming to improve the potency of the most potent inhibitors ME0385 and ME0386 (Figure 3.2).

The bioassay and the crystallographic results for these developed inhibitors ME0407 and ME0408 remain to be performed and to be added to this study. Most important would the evaluation of these inhibitors in virus-binding experiments to inhibit the attachment of Ad37 virion to HCE cells, and thus the prevention and the control of the infection can be assessed. Also, the structural studies of the modified N-acyl sialic acid will definitely add valuable information for further development of inhibitors of EKC-causing Ad37.

5. Experimental section

5.1 General chemistry

All reactions were carried out under inert atmosphere (N₂ gas). Sialic acid was purchased from Carbosynth. Silver triflate was purchased from Matrix Scientific. All other chemicals were purchased from Aldrich. Organic solvents were dried using the dry solvent system (Glass Contour Solvent Systems, SG Water USA) except CH₃CN and MeOH were dried over molecular sieves 3Å. Silica gel for chromatography was Merck (Silica gel 60) 70 – 230 mesh ASTM and solvents of analytical grade and deionized water of HPLC grade were used. TLC was performed on Silica gel 60 F254 (Merck) with deionized water of HPLC grade were used. Chromatography was Merck (Silica gel 60) 70 mesh ASTM. The volatile components were co-evaporated with toluene, CH₂Cl₂ and Et₂O repeatedly (3-5 times) to remove the remaining AcOH and afford compound 8a, and glycol 8b (32:1, 4.7 g) in quantitative yield. The product was used without additional purification, thus NMR data is not included. MS m/z (ES⁺) calcd. for C₁₂H₂₁NNaO₉ 346.1 [M+Na⁺], observed 346.2

5.2 Chemical synthesis

Methyl (5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (7).³⁹ Dowex 50WX (H⁺) (2.0 g) was washed with MeOH, mixed with anhydrous MeOH (200 mL), and N-Acetylneuraminic acid (3.0 g, 9.7 mmol) was added to the mixture portionwise at room temperature. The reaction mixture was stirred for 60 h† at rt, (TLC control: Rₚ=0.33 (7), CH₂Cl₂/MeOH, 8:2). The mixture was filtrated with Celite, and concentrated under vacuum to afford compound 7 (3.03 g) in quantitative yield. The product was used without additional purification, thus NMR data is not included. MS m/z (ES⁺) calcd. for C₁₂H₂₁NNaO₉ 346.1 [M+Na⁺], observed 346.2

Methyl (5-N-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-chloro-D-glycero-β-D-galacto-2-nonulopyranosyl)onate (8a).³⁹ Anhydrous MeOH (10 mL) was slowly added dropwise to AcCl (60 mL) at 0°C followed by AcOH 99% (32 mL). The resulting solution was transferred into flask containing compound 7 (3.0 g, 9.37 mmol) and maintained at 0 °C. The mixture was stirred at room temperature for 48 h, (TLC control: Rₚ=0.26 (8a and 8b) Tol/Acetonitrile, 2:1). The volatile components were co-evaporated with toluene, CH₂Cl₂ and Et₂O repeatedly (3-5 times) to remove the remaining AcOH and afford compound 8a, and glycol 8b (32:1, 4.7 g) in quantitative yield. The product was used without additional purification. Compound (8a) data:¹H NMR (CDCl₃): δ = 5.47 (dd, J = 7.2 and 2.3 Hz, 1H), 5.40 (dd, J = 10.8, 10.8 and 4.7 Hz, 1H), 5.20 - 5.15 (m, 1H), 4.41 (dd, J = 12.6 and 2.6 Hz), 4.34 (dd, J = 10.8 and 2.8 Hz, 1H), 4.25 - 4.15 (m, 1H), 4.06 (dd, J = 12.6 and 5.6 Hz, 1H), 3.87 (s, 3H, -OCH₃), 2.78 (dd, J₃=14.8 and J₄=4.8 Hz, 1H, H₃ax), 2.28 (dd, J₃ax=14.7 and J₄ax=11.3 Hz, 1H, H-3ax), 2.12 (s, 3H, -OCH₃), 2.07 (s, 3H, -OCOCH₃), 2.05 (s, 3H, -OCOCH₃), 2.04 (s, 3H, -OCOCH₃), 1.91 (s, 3H, -OCOCH₃) ppm. MS m/z (ES⁺) calcd. for C₂₀H₂₄Cl₂NO₁₂ 510.14 [M+H⁺], observed 509.80

Methyl (thiophenyl 5-N-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosyl)onate (9).³⁹ Compound 8 (4.7 g, 9.21 mmol) was dissolved in anhydrous CH₂Cl₂ (35 mL). Under dark condition, thiophenol (1.41 mL; 13.8 mmol, 1.5 eq) was added

† This is not the optimum conditions for this reaction! See the reference for the optimum condition.
and followed by DIPEA (2.4 mL; 13.8 mmol, 1.5 eq). The reaction mixture was stirred overnight (ca 20 h) at room temperature under dark, (TLC control: Rf=0.20 (9 and 8b), Tol/Acetone 2:1). The mixture was concentrated under vacuum. Purification by column chromatography (Tol/Acetone 2:1) afforded inseparable mixture of α/β anomers of 9 and glycal 8b (7:1:2, 2.6 g) in 48% yield. The product was used without additional purification. MS m/z (ES⁺) calcd. for C₂₀H₃₄NO₁₅S 582.2 [M+H⁺] and C₂₀H₃₅NaNO₁₂S 606.2 [M+Na⁺], observed 583.95 and 605.93 respectively.

**Methyl (thiophenyl 5-N,N-tert-butoxycarbonylacetamido-4,7,8,9-tetra-O-acetyl-2,3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylo)nate (10a).**²⁸ Compound 9 (2.6 g, 4.47 mmol), Boc₂O (2.9 g, 13.4 mmol, 3 eq), and DMAP (327 mg, 2.68 mmol, 0.6 eq) were dissolved in dry THF (73 mL). The mixture was refluxed for 3 h at ca 89 °C, (TLC control: Rf = 0.33 (10a, 10b), EtOAc/n-Heptane, 1:1). The mixture was cooled at room temperature, diluted with CH₂Cl₂, washed with HCl (0.5 M), NaHCO₃ solution and dried over MgSO₄. Purification by column chromatography (n-Heptane/EtOAc, step-elution 2:1 → 1:1) afforded inseparable mixture of α/β of compound 10a and glycal 10b (7:1:2, 2.4 g) in 80% yield that was used without additional purification. MS m/z (ES⁺) calcd. for C₃₁H₄₃N₃NaO₁₅S 706.2 [M+Na⁺], observed 706.1

**Methyl (thiophenyl 5-N-tert-butoxycarbonyl)-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylo)nate (11).**²⁸ Compound 10 (2.41 g, 4.13 mmol) was dissolved in MeOH (215 mL) and sodium methoxide (342 mg, 6.34 mmol, 1.54 eq) (0.03 M) was added at room temperature. The mixture was stirred for 4 h at room temperature, (TLC control: Rf=0.7 (11), CH₂Cl₂/MeOH, 10:1). The mixture was neutralized with drops of AcOH 99%. Purification by column chromatography (CH₂Cl₂/MeOH step-elution 99:7:0.3 → 99.5:0:5) and collection of a product with Rf=0.15 afforded α-anomer of compound 11 (1.03 g) in 27% yield over four steps. ²H NMR (CD₂OD): δ 7.57 – 7.53 (m, 2H, Ph), 7.45 – 7.39 (m, 1H, Ph), 7.33 – 7.39 (m, 2H, Ph), 3.81 (dd, J₉₉,₉₈= 9.4 and J₉₈,₉₇= 2.6 Hz, 1H, H-9a), 3.78 (m, 1H, H-8), 3.62 (s, 3H, CO₂CH₃), 3.56 – 3.52 (m, 3H, H-9b, H-7, H-4), 3.49 (t, J₉₉,₈=J₈₈,₇= 9.9 Hz, 1H, H-5), 3.34 (appear as bs, 1H, H-6), 2.83 (dd, J₉₃₆,₃₅= 12.8 and J₉₃₅,₃₄= 4.6 Hz, 1H, H₃₅₆), 1.85 (dd, J₉₃₅,₃₄= 12.8 and J₉₃₄,₃₃=11.1 Hz, 1H, H-3₃₄), 1.43 (s, 9H, -OC(CH₃)₃) ppm. ¹³C NMR (CD₂OD): 171.09 (CO), 159.53 (CO), 137.85 (Ph), 131.17 (Ph), 130.21 (C-2), 129.90 (Ph), 77.83 (C-6), 73.07 (C-8), 70.28 (C-7), 68.98 (C-4), 64.56 (C-9), 54.52 (C-5), 53.31 (OCH₃), 41.91 (C-3), 28.67 (OCO(CH₃)₃).

**Methyl (thiophenyl 5-N-tert-butoxycarbonyl-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (12).**²⁸ Compound 11 (1.03 g, 2.17 mmol) was dissolved in pyridine (40 mL) at 0 °C and Ac₂O (14 mL) was added on portions and the reaction stirred overnight at room temperature. (TLC control: Rf=0.17 (12), CH₂Cl₂/MeOH, 99:7:0.3). The reaction was monitored with LCMS for completion. The mixture was neutralized with MeOH (10 mL) and co-evaporated with toluene under vacuum. Purification by column chromatography (Tol/EtOH 20:1) afforded α-anomeric of compound 12 and additional 14 % of the corresponding elimination product (1.39 g) in quantitative yield that was used without additional purification. ²H NMR data for compound α-12 (CDCl₃): δ= 7.53-7.49 (m, 2H, Ph), 7.39-7.36 (m, 1H, Ph), 7.36-7.30 (m, 2H, Ph), 5.39 (dd, J= 7.6 and 1.9 Hz, 1H), 5.32-5.27 (m, 1H), 4.81 - 4.73 (ddd, J= 11.2, 11.2 and 4.5 Hz, 1H), 4.38 (dd, J= 12.3 and 2.8 Hz, 1H), 4.18 (dd, J= 12.1 and 5.3 Hz, 1H), 3.82 (dd, J= 10.7 and 1.8 Hz, 1H), 3.72-3.62 (m, 1H), 3.56 (s, 3H, -OCH₃), 2.82 (dd, J= 12.8 and 4.7 Hz, 1H, H-3eq), 2.13 (s, 3H, -OOCCH₃), 2.06 (s, 3H, -OOCCH₃), 2.03 (s, 3H, -OOCCH₃), 2.02 (s, 3H, -OOCCH₃),1.97 (appear as t, J= 12.2, 1H, H-3ax), 1.37 (s, 9H, OCO(CH₃)₃) ppm. MS m/z (ES⁺) calcd. for C₃₂H₄₄N₃NaO₁₅S 664.2 [M+Na⁺], observed 663.86

**Methyl (thiophenyl 5-ammonium-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate TFA salt (13).**²⁸ Compound 12 (1.36 g, 2.13 mmol) was dissolved in CH₂Cl₂ (19 mL) and 90% eq. TFA (8 mL) was added. The reaction stirred for 1.5 h at room temperature (TLC control: Rf=0.43 (13) Tol/EtOH 8:2). The mixture was co-evaporated with toluene, CH₂Cl₂ and Et₂O under vacuum to afford the ammonium salt (1.39 g) in quantitative yield that was used directly for coupling reaction. MS m/z (ES⁺) calcd. for C₂₄H₃₂N₂O₁₁S 542.1 [M+Na⁺], observed 541.9

**Methyl (thiophenyl 5-N-propanoyl-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (14).**²⁸ Compound 13 (1.39 g, 2.12 mmol) was dissolved in anhydrous CH₂Cl₂ (14 mL) and propanoic acid (190 μL, 2.57 mmol, 1.2 eq) was added followed by addition of HATU (980 mg, 1.2 mmol, 1.2 eq) of and DIPEA (1.3 mL, 7.4 mmol, 3.5 eq). The reaction
mixture was stirred at room temperature for 21 h (TLC control: Rf=0.2 (14) Tol/Acetone 4:1). Purification by column chromatography (Tol/Acetone 4:1) and collection of a product with Rf=0.2 afforded compound 14 (1.07 g) and tetramethyl urea (6:1) in 85% yield. \textsuperscript{1}H NMR (CDCl\textsubscript{3}): δ= 7.52-7.48 (m, 2H, Ph), 7.41-7.35 (m, 1H, Ph), 7.35-7.29 (m, 2H, Ph), 5.28 - 5.22 (m, 2H, H-7, H-8), 5.19 (d, J\textsubscript{NMe,5} = 10.1 Hz, 1H, -CONH), 4.89 - 4.81 (ddd, J\textsubscript{4,3ax}=11.5, J\textsubscript{4,5}= 10.3 and J\textsubscript{4,3eq}= 4.8 Hz, 1H, H-4), 4.37 (dd, J\textsubscript{9a,9b}= 12.4 and J\textsubscript{9b,9a}= 2.4 Hz, 1H, H-9b), 4.18 (ddd, J\textsubscript{9a,9b}= 12.4 and J\textsubscript{9a,5} = 5.1 Hz, 1H, H-9a), 3.99 (dd, J\textsubscript{5,5}=J\textsubscript{5,4} = 10.3 and J\textsubscript{5,5H}= 10.1 Hz, 1H, H-5), 3.89 (dd, J\textsubscript{5,5} = 10.4 and J\textsubscript{5,7}= 1.6 Hz, 1H), 3.55 (s, 3H, -OCH3), 2.80 (dd, J\textsubscript{3eq,3ax}= 12.8 and J\textsubscript{3eq,4} = 4.8 Hz, 1H, H-3eq), 2.12 (s, 3H, -OCOCH3), 2.07 (q, J\textsubscript{7,6} = 7.6 Hz, 2H, COCH\textsubscript{2}), 2.04 (s, 3H, -OCOCH3), 2.02 (s, 3H, -OCOCH3), 2.01 (bt, J\textsubscript{3eq,3ax}= 12.8, 1H, H-3ax), 1.99 (s, 3H, -OCOCH3), 1.95 (t, J\textsubscript{7,6} = 7.6 Hz, 3H, COCH\textsubscript{2}CH\textsubscript{2}), 1.73 (s, 3H, -OCOCH3), 1.57 (bt, J\textsubscript{3eq,4} = 12.8 and J\textsubscript{3eq,4} = 4.6 Hz, 1H, H-3eq), 2.14 (s, 3H, -OCOCH3), 2.09 (q, J\textsubscript{7,6} = 7.6 Hz, 2H, COCH\textsubscript{2}), 2.03 (s, 3H, -OCOCH3), 2.01 (s, 3H, -OCOCH3), 1.98 (bt, J\textsubscript{3eq,4} = 12.8 and J\textsubscript{3eq,4} = 4.6 Hz, 1H, H-3ax), 1.05 (t, J\textsubscript{7,6} = 7.6 Hz, COCH\textsubscript{2}CH\textsubscript{2}), ppm. MS m/z (ES\textsuperscript{+}) calcd. for C\textsubscript{27}H\textsubscript{36}NO\textsubscript{12}S \textsubscript{2} 620.18 [M+Na\textsuperscript{+}], observed 597.96 and 619.95 respectively.

**General method for glycosylation reaction** (17) and (18). Compound 14 (1.0 eq) was dissolved in a mixture of CH\textsubscript{3}CN/CH\textsubscript{2}Cl\textsubscript{2} (3:2) (35 mL/mmol). Crushed molecular sieves 3Å (1.5 g/mmole) was added followed by addition of glycosyl acceptor 15 or 16 (4.5 eq) and the mixture was stirred at room temperature under dark condition for 2 h. Solution of silver triflate (2.0 eq) in CH\textsubscript{3}CN was added dropwise to the mixture.\textsuperscript{4} The reaction mixture was stirred at room temperature under dark condition for 2 h. Solution of silver triflate (2.0 eq) in CH\textsubscript{3}CN was added dropwise to the mixture.\textsuperscript{4} The mixture was cooled to ca.-73 °C (-70 < t < -78), followed by addition of 1.0 M solution of I\textsubscript{2}Br (1.4 eq) in CH\textsubscript{2}Cl\textsubscript{2}.\textsuperscript{5} The reaction mixture was stirred at ca.-70 °C for 4.5 h, and was monitored by LCMS for completion. The reaction was quenched with DIPEA (6.0 eq) and kept stirred for 30 min at -70°C, and allowed to warm to room temperature. The mixture was filtrated with celite, washed with CH\textsubscript{2}Cl\textsubscript{2} and CH\textsubscript{3}CN and concentrated under vacuum. Purification by column chromatography afforded anomeric mixture.

**Methyl (3-bromo-propyloxy [5-N-propanoylalamide]-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-nonulopyranosyl]onate (17).** Column chromatography condition: elution with (Tol/EtOH 8:1) and collection of a product with Rf=0.25 afforded compound 17 (400 mg, 98%, α:β 22:3). The product was used without additional purification. \textsuperscript{1}H NMR data for α-17 (CDCl\textsubscript{3}): δ = 5.42-5.37 (m, 1H), 5.30 (dd, J\textsubscript{d,2} = 8.5 and 2.6 Hz, 1H), 5.10 (d, J\textsubscript{d,2} = 9.5 Hz, 1H, -CONH), 4.90 - 4.81 (m, 1H), 4.30 (dd, J\textsubscript{d,2} = 12.3 and 2.5 Hz, 1H), 4.13 (dd, J\textsubscript{d,2} = 10.6 and 2.6 Hz, 1H), 4.08 (dd, J\textsubscript{d,2} = 8.5 and 1.6 Hz, 1H), 3.89-3.82 (m, 1H), 3.81 (s, 3H, -OCH3), 3.80 (t, J\textsubscript{d,4} = 5.9, 2H, -OCH\textsubscript{2}CH\textsubscript{2}Br), 3.54 (t, J\textsubscript{d,4} = 6.5, 2H, -OCH\textsubscript{2}Br), 3.47 (dd, J\textsubscript{d,4} = 5.8 and 1.4 Hz, 1H), 3.43 - 3.36 (m, 1H), 2.57 (dd, J\textsubscript{d,4} = 12.6 and J\textsubscript{d,4} = 4.7 Hz, 1H, H-3eq), 2.14 (s, 3H, -OCOCH\textsubscript{3}), 2.13 (s, 3H, -OCOCH\textsubscript{3}), 2.08 (q, J\textsubscript{d,2} = 7.6 Hz, 2H, -COCH\textsubscript{2}), 2.03 (s, 3H, -OCOCH\textsubscript{3}), 2.00 (s, 3H, -OCOCH\textsubscript{3}), 1.94 (bt, J\textsubscript{d,ax}=J\textsubscript{d,ax}= 12.6 Hz, 1H, H-3ax), 1.99 (s, 3H, -OCOCH\textsubscript{3}), 1.05 (t, J\textsubscript{d,2} = 7.6, 3H, CH\textsubscript{3}CH\textsubscript{2}CH\textsubscript{3}) ppm. MS m/z (ES\textsuperscript{+}) calcd. for C\textsubscript{28}H\textsubscript{36}BrN\textsubscript{16}O\textsubscript{13} 621.1 [M+H\textsuperscript{+}], observed 613.89 and 635.89 respectively.

**General method for synthesis of azido derivatives (19) and (20).** Compound 17 or 18 (1.0 eq) was dissolved in DMSO (40 mL/mmol) and sodium azide (6.0 eq) was added followed by addition of TBAI (2.0 eq) portionwise. The reaction was stirred at room temperature for 6 h (TLC control: Rf=0.3 (19 or 20) Tol/EtOH 8:1). The reaction was monitored with LCMS for completion and the mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2}, washed with water and brine water, dried over MgSO\textsubscript{4}, filtered and concentrated under vacuum. Purification by column chromatography afforded the corresponding azido derivatives in quantitative yields.

\textsuperscript{4} This amount of the solvent was deducted from the total amount needed for the reaction.

\textsuperscript{5} See note above!
Methyl (3-azido-propoxy [5-N-propanoylamide]-4,7,8,9-tetra-O-acetyl 3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (19). Column chromatography condition: elution with (Tol/CH₂Cl₂/MeOH 8:2:0.5) and collection of a product with Rf=0.15 afforded compound 19 (419 mg, quantitative yield). The product was used without additional purification, thus NMR data is not included. MS m/z (ES⁺) calc'd. for C₂₂H₃₇N₂O₁₃ 589.2 [M+H⁺] and C₂₄H₃₉N₄NaO₁₃ 611.2 [M+Na⁺], observed 588.9 and 610.9 respectively.

Methyl (2-azidoethoxy [5-N-propanoylamide]-4,7,8,9-tetra-O-acetyl 3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (20). Column chromatography condition: elution with (Tol/CH₂Cl₂/MeOH 10:2:0.5) and collection of a product with Rf=0.10 afforded compound 20 (366 mg, quantitative yield). The product was used without additional purification, thus NMR data is not included. MS m/z (ES⁺) calc'd. for C₂₃H₃₅N₂O₁₃ 575.2 [M+H⁺] and C₂₅H₄₇N₄NaO₁₃ 597.2 [M+Na⁺], observed 575.0 and 597.0 respectively.

General method for deacetylation of azido derivatives (19) and (20). Compound 19 or 20 (1.0 eq) was dissolved in MeOH (70 mL), and sodium methoxide (0.03 M) (3.9 eq) was added, the mixture was stirred at room temperature for 3 h. (TLC control: Rf=0.2 (21) or 22) Tol/CH₂Cl₂/MeOH 3.5:6:0.5) and was monitored with LCMS for completion. The reaction mixture was neutralized with drops of acetic acid (99%), and concentrated under vacuum. Purification by column chromatography afforded α-anomer of 21 or 22.

Methyl (3-azido-propoxy [5-N-propanoylamide]-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (21). Column chromatography condition: step-elution with (Tol/CH₂Cl₂/MeOH 3.5:6:0.5 → 3.6:1) and collection of a product with Rf=0.21 (Tol/CH₂Cl₂/MeOH 3.6:1) afforded α-21 (157 mg, 55%) over three-steps. ¹H NMR (CD₃OD): δ = 3.89-3.80 (m, 3H, -OCH₂-), 2.95-2.83 (m, 3H, -CH₂-), 3.64-3.62 (m, 2H, H-9a, H-4), 3.58 (dd, J₆,₅ = 10.2 Hz, 1H, H-5), 3.63-3.60 (m, 2H, H-9a, H-4), 3.58 (dd, J₆,₅ = 10.2 and J₅,₆≈ 6.0 Hz, 1H, H-6), 3.49 (dd, J₅,₆≈ 8.9 and J₆,₇≈ 1.6 Hz, 1H, H-7), 1.14 (t, J = 7.6 Hz, 3H, -COCH₂-), 1.76 (dd, J₃,₅≈ 12.8 and J₅,₆≈ 11.6 Hz, 1H, H-3ax), 1.14 (t, J = 7.6 Hz, 3H, -COCH₂-) ppm. ¹³C NMR (CD₃OD): 178.99 (CO), 171.08 (CO), 100.20 (C-2), 75.01 (C-6), 72.43 (C-8), 70.18 (C-7), 68.42 (C-4), 64.69 (C-9), 62.07 (C-21), 53.68 (C-5), 53.39 (OCH₃), 48.78 (-CH₂N₃), 41.76 (C-3), 30.16 (-COCH₂), 30.13 (-CH₃-), 10.28 (-CH₃) ppm. MS m/z (ES⁺) calc'd. for C₁₆H₂₃NO₇ 421.19 [M+H⁺] and C₁₆H₂₃NO₇NaO₇ 443.18 [M+Na⁺], observed 421.00 and 443.00 respectively.

Methyl (2-azidoethoxy [5-N-propanoylamide]-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (22). Column chromatography condition: step-elution with (Tol/CH₂Cl₂/MeOH 3.5:6:0.5 → 3.6:1) and collection of a product with Rf=0.18 (Tol/CH₂Cl₂/MeOH 3.6:1) afforded α-22 and additional impurities, another column condition: elution with (CH₂Cl₂/MeOH 9:1) and collection of a product with Rf=0.23 afforded pure α-22 (120 mg, 46%) over three-steps. ¹H NMR (CD₃OD): δ = 4.00-3.94 (m, 1H, -OCH₂-), 3.85 (s, 3H, OCH₃), 3.84-3.80 (m, 2H, H-8, H-9b), 3.77 (t, J₆,₄ = 6.0 Hz, 1H, H-5), 3.72-3.67 (dd, J₄,₃ax≈ 11.6, J₆,₄≈ 10.4, J₄,3eq≈ 4.6 Hz, 1H, H-4), 3.66-3.60 (m, 2H, H-9a, -OCH₂-), 3.57 (dd, J₆,₅≈ 10.2 and J₅,₆≈ 1.6 Hz, 1H, H-7), 3.35-3.32 (3m, 1H, -CH₂N₃), 3.35-3.32 (2m, 1H, CH₂=CH), 2.72 (dd, J₃,₅ax≈ 12.8 and J₅,₆≈ 4.6 Hz, 1H, H-3eq), 2.27 (q, J = 7.6 Hz, 2H, -COCH₂-), 1.76 (dd, J₃,₅ax≈ 12.8 and J₅,₆≈ 11.6 Hz, 1H, H-3ax), 1.14 (t, J = 7.6 Hz, 3H, -COCH₂-) ppm. ¹³C NMR (CD₃OD): 179.01 (CO), 170.73 (CO), 100.19 (C-2), 75.09 (C-6), 72.36 (C-8), 70.17 (C-7), 68.40 (C-4), 64.72 (C-9), 64.47 (-OCH₂-), 53.64 (C-5), 53.43 (CH₃), 51.73 (-CH₂N₃), 41.64 (C-3), 30.16 (-COCH₂), 10.28 (-CH₃) ppm. MS m/z (ES⁺) calc'd. for C₁₅H₂₂NO₇ 407.18 [M+H⁺] and C₁₅H₂₂NO₇NaO₇ 429.16 [M+Na⁺], observed 407.09 and 429.02 respectively.

General method for trivalent sialic acid synthesis of (23) and (24). Azido derivative compound (21) or (22) (3.7 eq) was dissolved in THF/H₂O (1:1) (81 mL/mmol), and tripargylamine (1.0 eq) was added followed by addition of CuSO₄ (0.9 eq) and sodium ascorbate (0.9 eq). The mixture was stirred at 50 °C for 3 h, and at room temperature for additional 18 h. The reaction was monitored with LCMS for completion. THF solvent was evaporated under vacuum and the crude was freeze dried. Purification by HPLC (A: aq. 0.005% CF₃COOH in H₂O, B: aq. 0.005% CF₃COOH in CH₂CN, organic phase gradient 7% → 25%). The collected fractions were freeze dried to afford the trivalent methyl ester of the sialic acid derivatives (23 or 24) in 35-50% yields.
Tris-[2-O-(methyl-5-N-propanoylamide-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)-onate]-3-oxopropyl-(1H-1,2,3-triazole-4-yl)-methyl]amine. (23). Yield: compound 23 (45 mg, 34%). 1H NMR (CD3OD): δ= 8.03 (s, 3H, 3xArCH), 7.89 (d, J=8.5 Hz, ca 3H, 3xNH), 4.50 (t, J= 6.5 Hz, 6H, 3x-CH2ArN), 3.86-3.73 (m, 18H, 3x-OCH2Br, 3xH-9b, -N(CH2)5, 3xH-8, 3xH-5), 3.81 (s, 9H, 3x-OCH3), 3.70-3.58 (m, 6H, 3xH-4, 3xH-9b), 3.56 (dd, J= 10.4 and J= 1.3 Hz, 3H, 3xH-6), 3.47 (dd, J= 8.9 and J= 1.3 Hz, 3H, 3xH-7), 3.43-3.35 (m, 3H, 3x-OCH2CH2) ppm. 13C NMR (CD3OD): 178.92 (CO), 170.88 (CO), 143.82 (Ar-C) 126.16 (ArCH2), 100.12 (C-2), 75.01 (C-6), 72.44 (C-8), 70.22 (C-7), 68.44 (C-4), 64.72 (C-9), 61.73 (-OCH2-), 53.67 (C-5), 53.52 (OCH2), 49.28 (N(CH2)3), 49.07 (ArN-CH2), 41.75 (C-3), 31.28 (-COCH3), 30.17 (-CH2-), 10.32 (-CH3) ppm. MS m/z (ES+) calc. for C57H96N13O27 1392.6 (M+H+) and C57H95N13NaO27 1414.6 (M+Na+) observed 1392.50 and 1414.5 respectively.

Tris-[2-O-(methyl-5-N-propanoylamide-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)-onate]-2-oxoethyl-(1H-1,2,3-triazole-4-yl)-methyl]amine. (24). A trace of copper was removed after HPLC then purification by column chromatography was performed (CH3CN/H2O 1:1), and a product of R=0.7 was collected to afford 24 (60 mg, 50%). 1H NMR (CD3OD): δ= 8.04 (s, 3H, 3xArCH), 4.50 (t, J= 5.1 Hz, 6H, 3x-CH2ArN), 4.23-4.16 (m, 3H, 3x-OCH2Br), 3.92-3.86 (m, 3H, 3x-OCH2), 3.84-3.74 (m, 15H, 3xH-9b, -N(CH2)5, 3xH-8, 3xH-5), 3.72 (s, 9H, 3x-OCH3), 3.67-3.59 (m, 6H, 3xH-4, 3xH-9b), 3.57 (dd, J= 10.4 and J= 1.3 Hz, 3H, 3xH-6), 3.47 (dd, J= 8.9 and J= 1.3 Hz, 3H, 3xH-7), 2.58 (dd, J= 12.4 and J= 4.6 Hz, 3H, 3xH-3eq), 2.25 (q, J= 7.6 Hz, 6H, 3x-CH2CH2) ppm. 13C NMR (CD3OD): 178.88 (CO), 170.43 (CO), 145.08 (Ar-C), 126.66 (ArCH2), 100.23 (C-2), 75.04 (C-6), 72.15 (C-8), 69.97 (C-7), 68.29 (C-4), 64.73 (C-9), 63.95 (-OCH2-), 53.60 (C-5), 53.45 (OCH3), 51.43 (N(CH2)3), 49.07 (ArN-CH2), 41.52 (C-3), 31.15 (-COCH3), 22.97 ppm. MS m/z (ES+) calc. for C56H88N13O27 1350.59 [M+H+] observed 1350.43

General method for saponification of the methyl ester (23 and 24). Trivalent methyl ester derivatives of sialic acid (23 or 24) (1.0 eq) was dissolved in MeOH (135 mL/mmol), 1.0 M solution of LiOH (9.0 eq) in water was added and the mixture was stirred at room temperature for 9 h. The reaction was monitored with LCMS for completion, and the mixture was neutralized with Dowex 50W (H+). The solvent was evaporated under vacuum and the crude was eluted on C-18 plug with H2O and freeze dried to afford 25 (ME0407) or 26 (ME0408) in 72-75% yields.

Tris-[2-O-(methyl-5-N-propanoylamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-3-oxopropyl-(1H-1,2,3-triazole-4-yl)-methyl]amine. (25) (ME0407). Yield: Compound 25 (27 mg, 75%). [α]D -0.76 (c 0.48, H2O). 1H NMR (D2O): δ= 8.31 (s, 3H, 3xArCH), 4.63-4.53 (m, 12H, 3x-CH2ArN, N(CH2)3), 3.82 (dd, J= 11.8 and J= 2.4 Hz, 3H, 3xH-9b), 3.83-3.74 (m, 12H, 3x-OCH2Br, 3xH-8, 3xH-5), 3.73-3.67 (m, 3xH, 3H-4), 3.67 (dd, J= 10.4 and J= 1.5 Hz, 3H, 3xH-6), 3.60 (dd, J= 11.8 and J= 8.9 Hz, 3xH-9a), 3.53 (dd, J= 8.9 and J= 1.5 Hz, 3H, 3xH-7), 3.50-3.42 (m, 3H, 3x-OCH2CH2), 2.69 (dd, J= 12.4 and J= 4.5 Hz, 3H, 3xH-3eq), 2.29 (q, J= 7.6 Hz, 6H, 3x-CH2CH2), 2.25-2.15 (m, 6H, 3x-CH2CH2) ppm. 13C NMR (D2O): 178.87 (COHN), 173.77 (COOH), 136.66 (Ar-C) 128.98 (ArCH2), 100.86 (C-2), 73.47 (C-6), 72.34 (C-8), 69.04 (C-7), 68.66 (C-4), 63.47 (C-9), 61.82 (-OCH2-), 52.52 (C-5), 48.52 (N(CH2)3), 47.57 (ArN-CH2), 40.83 (C-3), 30.17 (-COCH3), 30.10 (-CH3) ppm. MS m/z (ES+) calc. for C56H88N13O27 1350.59 [M+H+] observed 1550.28.

Tris-[2-O-(5-N-propanoylamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-2-oxoethyl-1H-1,2,3-triazole-4-yl]-methyl]amine. (26) (ME0408). Yield: Compound 26 (19 mg, 72%). [α]D -14.34 (c 0.46, H2O). 1H NMR (D2O): δ= 8.17 (s, 3H, 3xArCH), 4.64 (bs, 6H, 3x-CH2ArN), 4.20-4.07 (m, 9H, 3x-OCH2Br, -N(CH2)3), 3.93-3.87 (m, 3H, 3x-OCH2), 3.82 (dd, J= 11.7 and J= 2.4 Hz 3H, 3xH-9b), 3.75 (dd, J= 8.9, J= 5.6 Hz, J= 2.4 Hz 3H, 3xH-8), 3.72 (t, J= 10.1 Hz, 3H, 3xH-5), 3.69-3.63 (m, 3H, 3xH-4), 3.63-3.56 (m, 6H, 3xH-6, 3xH-9a), 3.51 (dd, J= 8.9 and J= 1.6 Hz, 3H, H-7), 2.66 (dd, J= 12.3 and J= 4.6 Hz, 3H, 3xH-3eq), 2.28 (q, J= 7.6 Hz, 6H, 3x-CH2CH2), 1.74 (appear as t, J= 12.3 Hz, 3H, 3xH-3ax), 1.10 (t, J= 7.6 Hz, 9H, 3x-COCH2CH2) ppm. 13C NMR
lovely country.

me a place to accomplish my master's degree in this

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