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Dipartimento di Scienze Biomediche Chirurgiche e Odontoiatriche

Synthesis and Biological Evaluation of Some Unsaturated Neuraminic Acid Analogues as Potential Sialidase Inhibitors

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Summery

This work represents a part of a larger research project directed to study the correlation between the different structures of unsaturated Neu5Ac derivatives and their inhibitory activity against viral, bacterial and mammalian sialidases. So, interested in finding the general features to define a potent and selective inhibitor of a particular kind of sialidase, we have set up many rapid, simple and efficient protocols to introduce new substituents into the DANA structure. In this thesis we accomplish:

*- the synthesis of new C-4a aminated DANA derivatives (**1a-d**; **2a-d**);*

*- the first synthesis of N-perfluorinated C-4 epimers of DANA (**11b-d**), clarifying also their mechanism formation and the key role of the oxazoline derivative **16**;*

***-** the first Ritter-like reaction performed on Neu5Ac derivatives, to obtain compounds **18a-d**;*

***-** The extension of the Ritter reaction leading to formulate a general nucleophilic substitution at C-4 position of e Neu5Ac glycals, synthesizing a great number of compounds by the rapid introduction of alcohols, thiols, sulfonamides and alogens;*

-The set up of a general method to introduce nucleophiles at C-2 position of Neu5Ac analogues to obtain new 3,4 unsaturated alkylglycosides, to be biologically tested, or to be used as synthetic intermediate to functionalize C-3 and C-4 positions of neuraminic acid derivatives;

*- The preliminary fluorimetric sialidase inhibition assays of part of the obtained compounds, more precisely of glycals **1a-d**, **2a-d**, **11a-d** and **18b-d**, against Vibrio cholerae, Newcastle disease virus, Clostridium perfringens and mammalian Neu3 sialidases. We obtain interesting results especially regarding the NDV neuraminidase inhibition data, suggesting the possibility to design a selective NDV NA inhibitor by tuning the substituent at C-5 position.*

1. INTRODUCTION

1. Introduction

Sialic acids (sias), in particular neuraminic acid (Neu5Ac), a family of nine-carbon sugars, are often the terminal acidic monosaccharides of cell membrane glycoconjugates of various living organisms and can modulate many biological processes including cell-to-cell recognition, cell adhesion and inflammation.[1] Thus, their removal, catalyzed by a sialidase, influences these processes through changes in glycoconjugate conformation and creations or losses of binding sites of functional molecules. The sialidases are widespread in a variety of microorganisms, also in ones that do not synthesize the sias themselves. This fact led to the discovery of their role in the pathogenesis of numerous microbial diseases[2]. Moreover, also the viruses have specific genomic segments encoding for sialidases (neuraminidases, NAs) and these proteins play a specific and critical role to spread the virus infection. Furthermore, these enzymes widely exist also in vertebrates and recent research on mammalian sialidases has proven the great importance of these enzymes in several cellular functions as the lysosomal catabolism and the regulation of cell proliferation[3]. For these reasons, in recent years, sialidases have been the subject of an intense research to discover potential therapeutic agents acting as sialidase inhibitors. This research started from the depth study of the sialidases hydrolysis mechanism that highlights the presence of highly conservated binding tool in the active site[4]. In fact, in all cases, the active site of exo-sialidase enzymes contains three acidic amino acids, a tyrosine, and three arginines, where the key residues involved in catalysis are an aspartic acid, a glutamic acid, and the tyrosine residue (**Figure 1, A**).

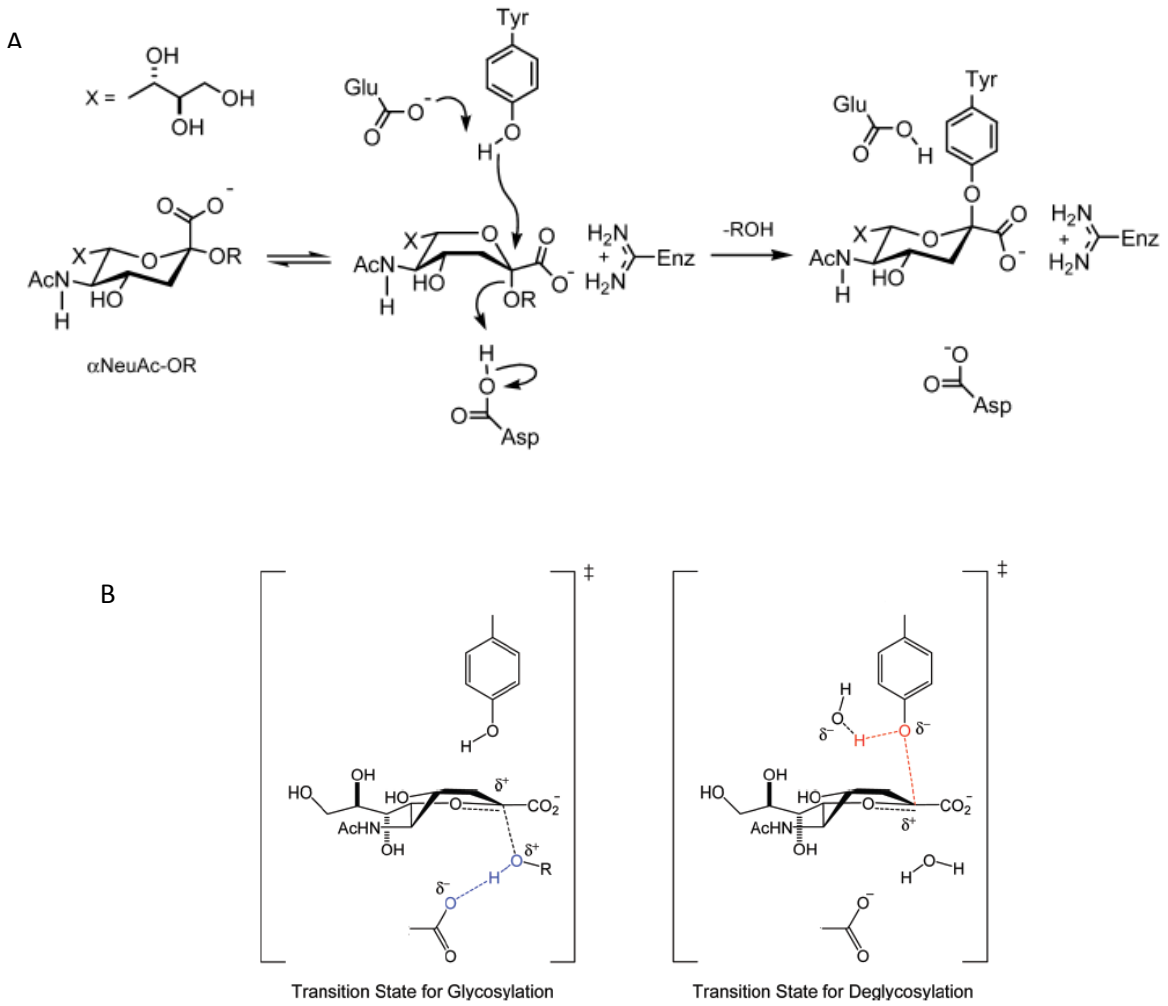


Figure 1: *A) Catalytic mechanism of sialidase hydrolysis; **B**) Oxocarbenium ion intermediate*

This hydrolysis passes through an oxocarbenium ion intermediate (**Figure 1**, **B**) that is mimicked by the generic sialidase inhibitor 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (Neu5Ac2en, DANA). This compound inhibits the majority of sialidases in the micromolar range, riproducing the flattened half-chair hydrolysis transition state. Consequently, the DANA structure has become the most common starting scaffold in the research for improved sialidase inhibitors.[5]

As a part of a larger project directed to expand the structure/activity relationship comprehension of sialidase inhibitors, we herein report new simple procedures to easily synthesize novel DANA derivatives possibly useful as sialidase inhibitors.

In particular, we focus on the development of straight synthetic pathways to rapidly modify positions 4 and 5 of DANA analogues, that are known to be fundamental for the enzyme-substrate interaction; and on the synthesis of new 3,4-unsaturated Neu5Ac conjugates possibly useful both as neuraminidase inhibitors and as synthetic intermediate to functionalize positions 3 and 4 of the monosaccharide structure. Furthermore, the preliminary biological assays of the newly synthesized compounds are performed on the sialidases of different sources to evaluate their inhibition activity.

In the following paragraphs, after a general introduction about the sialic acid derivatives and a short description of some particular viral, bacterial and mammalian sialidases, we will discuss all the experimental results obtained during my Ph.D. study.

1.2 Sialic acids

According to the IUPAC nomenclature[6], the original name of neuraminic acid is 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (abbreviated as Neu). The three basic structures of this family derivatives of Neu are: *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc) and KDN.

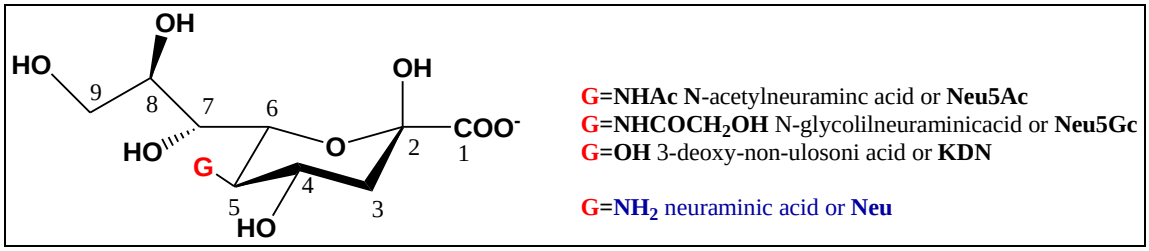


Figure 1.1 *Main derivatives of sialic acids*

The carbon atoms of sias are numbered consecutively this way: a carboxyl group receives locate 1, and the anomeric position is 2.

As show in **Figure 1.1** the most common member of this family is Neu5Ac, a nine carbon carboxylated monosaccharide with D-*glycero*-D-*galacto* configuration.[7]

In nature, sialic acids are generally found as part of glycoconjugates, often as the terminal components, being commonly α(2,3)- or α(2,6)-linked to hexoses, or α(2,8)- linked to other sialic acids.

The biosynthesis of Neu5Ac[8] (**Figure 1.2**) from simple hexoses in mammals is achieved from *N*-acetyl-D-mannosamine, which is phosphorylated to ManNAc-6-phosphate (ManNAc-6-P) by the MNK kinase catalytic domain. ManNAc-6-P is then converted into Neu5Ac-9-P by a specific aldolase that must be dephosphorylated by a specific phosphatase, giving free Sias in the cytoplasm. Neu5Ac is activated in the nucleus of cells, by cytidine monophosphate (CMP)-sialate synthetase, to give the nucleotide sugar CMP-Neu5Ac. Subsequently, Neu5Ac, is transferred to acceptor substrates in the Golgi apparatus by a sialyltransferase. Further modifications (such as *O*-acetylation) of the glycosidically-bound sialic acids also occur in the Golgi apparatus before transportation to the outer cell membrane as terminal residue of glycoconjugates. Due to their external position, sialic acids can play several cellular recognition roles, generally acting either as receptors, or as a mask for recognition sites.

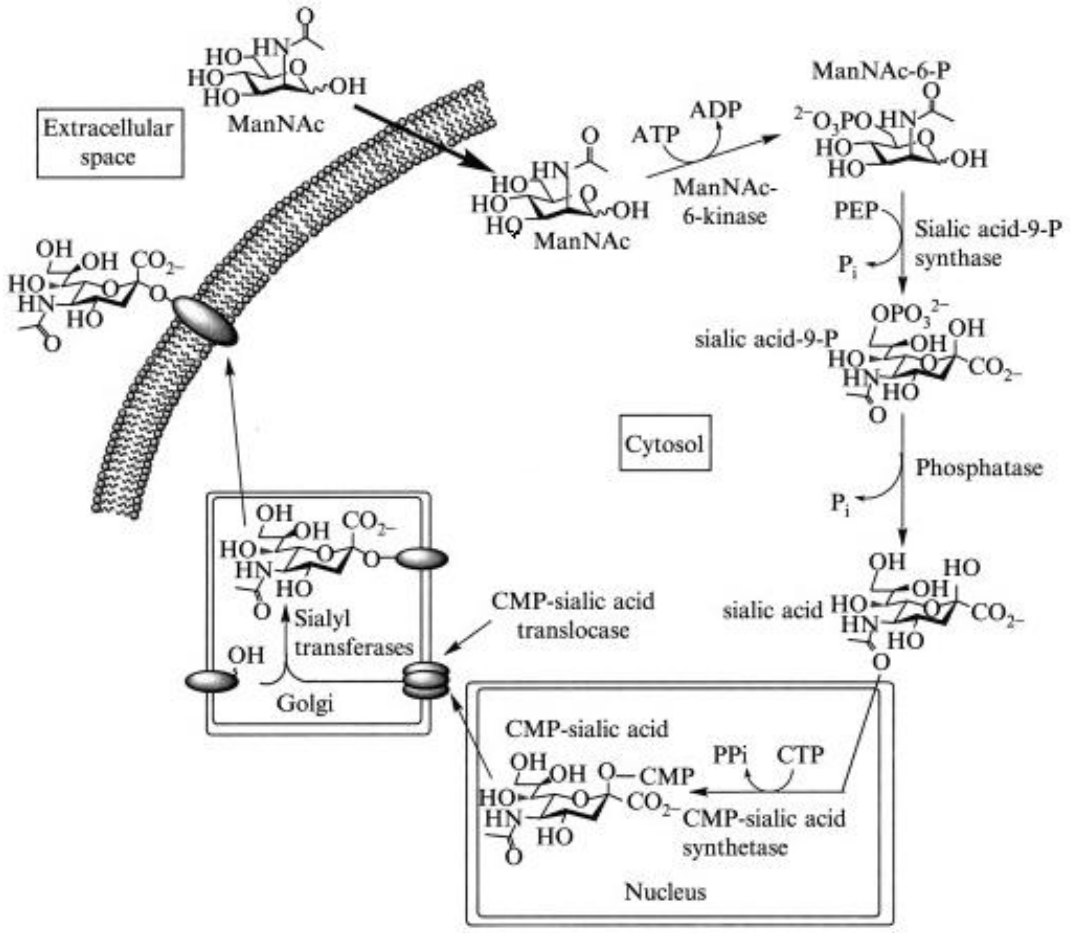


Figure 1.2: *Neuraminic acid biosynthesis*

Indeed, there are many viral and bacterial proteins able to recognize the terminal sialic acid residue involved in the infection processes[9], as viral hemagglutinins[10] and bacterial adhesins[11]. Moreover, several mammalian proteins that can bind sialic acid can affect endogenous biological functions, as lectins[12] and selectines[13].

In detail, sialic acid-recognising lectins, in conjunction with their sialic acid ligands, play an important role in blood circulation, cell adhesion, differentiation and proliferation, in the activation of the immune system and are also implicated in various disease such as cancer, inflammatory, autoimmune and genetically-related disorders.

Finally, we have to consider the principle enzymes involved in sialic acid catabolism, the sialidases, that catalyze the removal of sialic acid from the glycoconjugate, so modulating various biological processes through changes in

conformation and creation or loss of binding sites of functional molecules. These enzymes exist widely in vertebrates and also in a variety of microorganisms and recent research on mammalian sialidases has underlined the great importance of these enzymes in various cellular functions, including lysosomal catabolism; whereas microbial sialidases appear to play roles limited to nutrition and pathogenesis.

1.3 Sialidases

A great attention has been directed to a wide group of sialic acid-recognising proteins: the sialidases, otherwise known as the neuraminidases, that cleave the glycosidic linkage between a terminal sialic acid residue and the penultimate sugar of glycoconjugates, generally a D-galactose (Gal), a *N*-acetyl-D-galactosamine (GalNAc) or another sialic acid, thus representing the key enzymes for catabolism of sialic acid-containing molecules. These enzymes are divided in exosialidases[14], (**Figure 1.3**); in endosialidases[15] for the specific cleavage of linkages within α(2,8)-polysialic acid; and, finally, in *trans*-sialidases[16], that catalyze both the hydrolysis of sialic acids and transfer to another sugar.

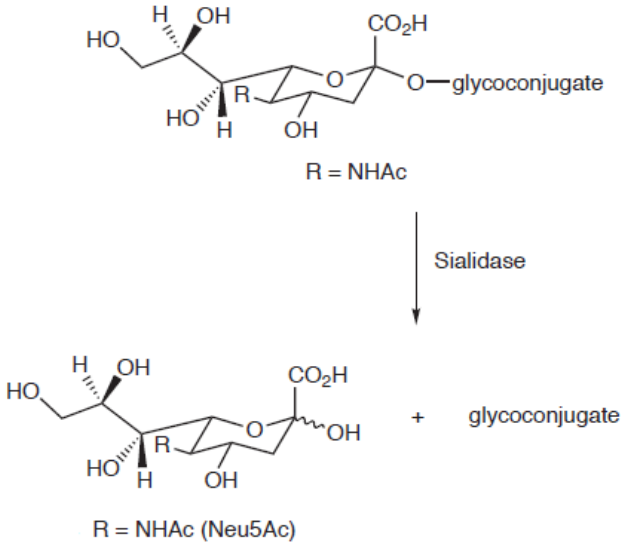


Figure 1.3: *Sialidase hydrolysis of the terminal neuraminic acid residue from a glycoconjugate*

Exosialidases cleave naturally occurring α(2,3), α(2,6) and α(2,8) ketosidic linkages[17], as shown in **Figure 1.4** and, generally, for microbial and animal exosialidases, α(2,3) linkages are cleaved at the highest rate.

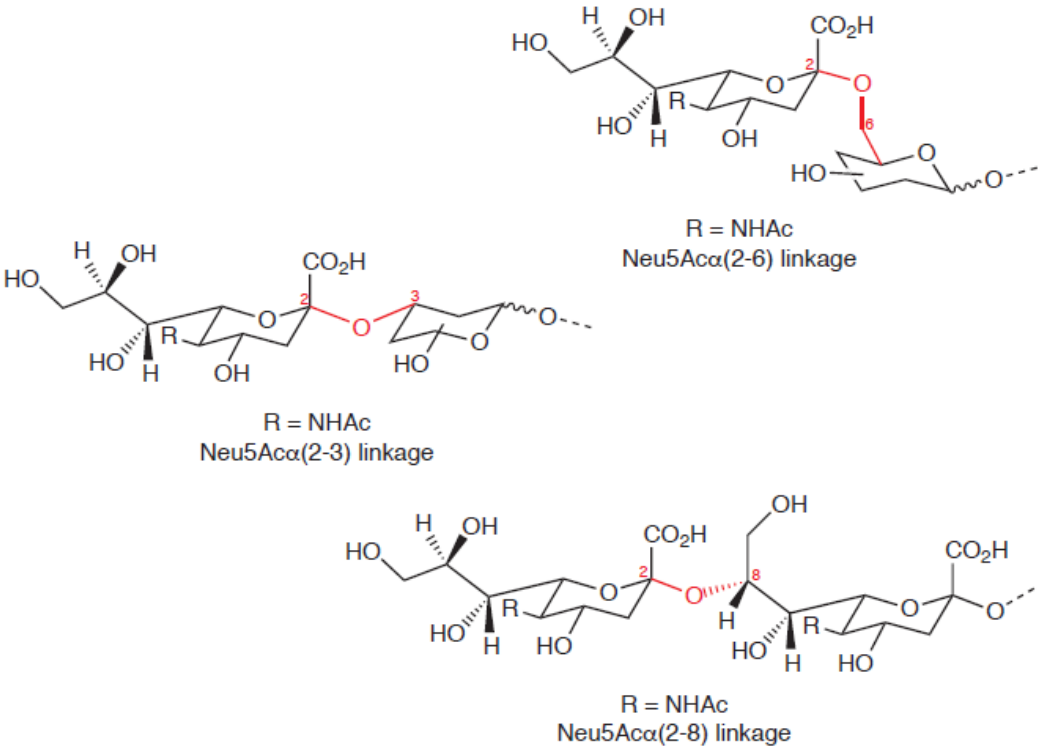


Figure 1.4: *Glycosidic linkage of neuraminic acids*

The susceptibility of a sialosyl glycoside to sialidase cleavage differs mainly for the kind of the ketosidic linkage, the sialic acid structure and the nature of the aglycone. In fact, it is know that *O*-Acetylation of the sialic acid generally reduces susceptibility to cleavage[18], and *N*-acetylneuraminic acids are more readily cleaved compared with *N*-glycolyl derivatives[19].

Sialidases are widespread in nature and they are present in viruses[20, 21], bacteria[22], protozoa[16], mycoplasma[23] and fungi[24] as well as in higher organisms such as birds and mammals[25] with different roles. To report a brief overview of the different sialidase kinds, in mammals four types of sialidases, encoded by different genes, have been identified and characterized to date, designated as Neu1, Neu2, Neu3 and Neu4. They have different cellular localization, in particular, are cytosolic (Neu2)[26], bound to the plasma membrane

(Neu3)[27], lysosomal (Neu1)[28], mitochondrial (Neu4) and are involved in catabolism of sialic acid-containing molecules and receptor modification to alter cellular functions (degradation in lysosomes, myoblast and neuronal differentiation, apoptosis, adhesion). As a virulence factor, sialidases can play a variety of roles: for example, the influenza virus neuraminidase catalyzes the release of viral progeny from host cells[29], and assists the virion particle movement through the respiratory tract by reducing the viscosity of the mucosal layer. While, microbial sialidases are able to scavenge sialic acid from their hosts and to use it as a carbon energy source[2], while in pathogenic species are often involved in infection processes[30].

As instance, *Clostridium perfringens*, the bacterium responsible for gas gangrene, secretes a sialidase employed to destroy the first line of the host defense, by cleaving the terminal sialic acid residues on host sialoglycoconjugates, paving the way for other bacterial enzymes to destroy the underlying host structures[31].

Some pathogenic bacteria, such as *Vibrio cholerae*, use neuraminidases to unveil receptors and to permit the passage of toxins across host membranes[32]. Finally, in protozoa, the *trans*-sialidase from the parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease, transfers sialic acids from host sialoglycoconjugates onto the parasite's own surface glycoproteins, aiding the evasion of the host immune system[33].

Thus, we have reported many varieties of sialidases that differ in size, substrate specificity, and the conditions needed for their optimum activity and, furthermore, a comparison of the primary structure of these enzymes revealed a low overall sequence identity, of generally less than 30%.

However, the x-ray crystal structure of sialidases from various strains of influenza virus, from the bacteria *Vibrio cholerae*[34], *Salmonella typhimurium*[35], and *Micromonospora viridifaciens*[36], from the leech *Macrobdella decora*[37], and from the parasites *Trypanosoma rangeli*[38] and *Trypanosoma cruzi*[39], has revealed that, despite their low overall sequence identity, neuraminidases share common features within the catalytic domain[40]. In fact, the catalytic site of all sialidases is

at the centre of a canonical domain consisting of six four-stranded antiparallel β-sheets folded into a propeller shape. Larger sialidases (over 60 kDa) contain multiple copies of this catalytic domain, or extra carbohydrate-binding domains, as the influenza virus neuraminidase, that is a tetramer consisting of four identical canonical catalytic domains linked by disulfide bonds[41]. Furthermore, some sialidases contain lectin-like binding domains, which are probably involved in anchoring the sialidase while it performs its catalytic function, and may also increase the catalytic efficiency of the sialidase when presented to multivalent substrates at cell surfaces[34]. There are also many conserved residues within the active site, which are believed to be important for sialoside cleavage.

The mechanism of sialidase catalysis has been studied in detail for influenza virus neuraminidase using NMR spectroscopy[42], deuterium isotope effects and computational molecular modeling techniques[43]. An S_N1-like mechanism for sialoside cleavage, involving the formation of an intermediate sialosyl cation, proposed by von Itzstein and coworkers[43]. A conserved arginine residue, which is protonated at optimum pH, is postulated to be involved in initial binding of the substrate, inducing the sialic acid to distort from a $^2C_5$ chair to a boat conformation (**Figure 1.5**). This distortion facilitates the cleavage of the glycosidic bond through the participation of the endocyclic oxygen atom, producing the sialosyl cation intermediate. Then, the nucleophilic attack of an activated water molecule to this intermediate produces the free sialic acid in the α-configuration, which rapidly mutarotates to the more stable β-configuration.

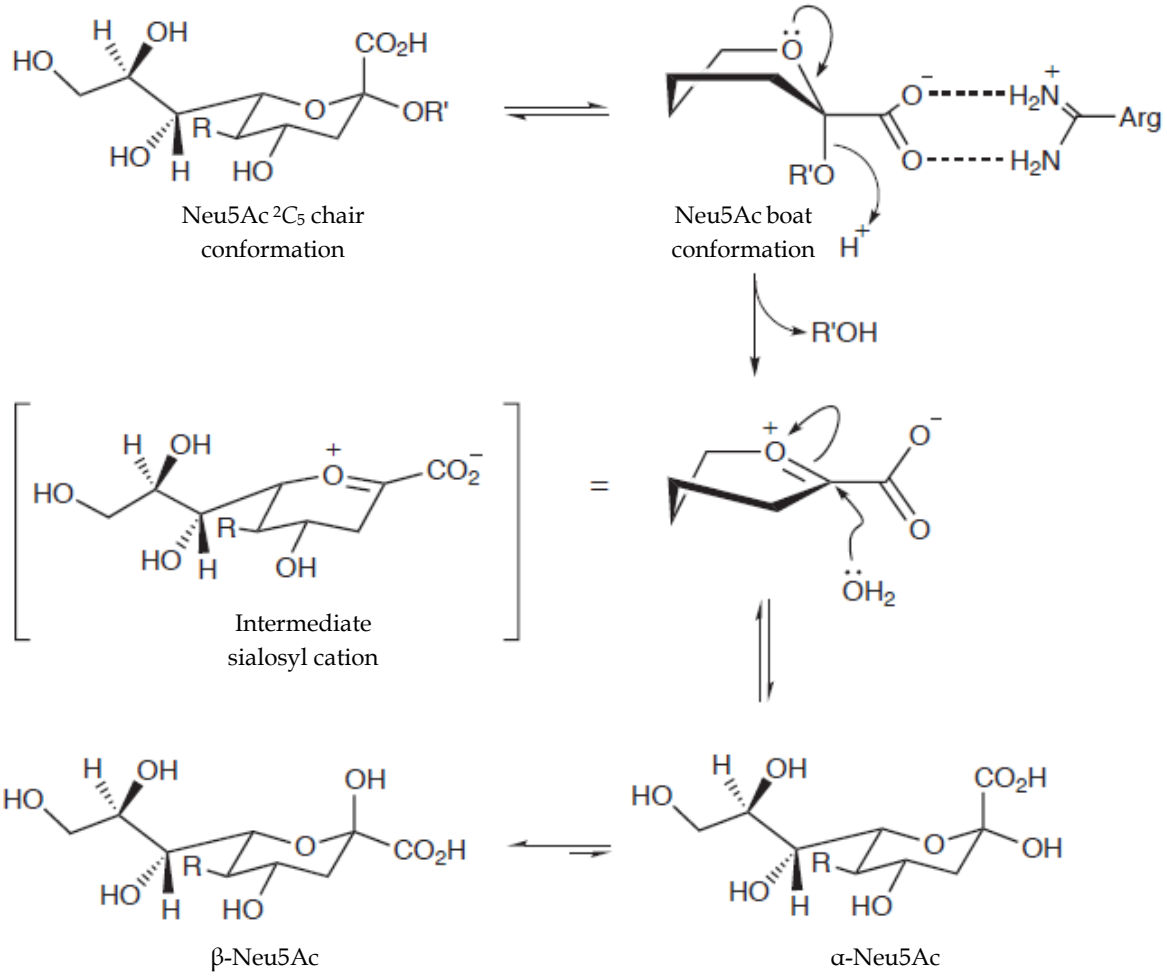


Figure 1.5: *Sialidase hydrolysis mechanism*

It is expected that this mechanism for sialoside hydrolysis is common to many other neuraminidases of viral and bacterial sources due to conserved residues in the active site. This hypothesis is supported by the finding that, like influenza virus NA, neuraminidases from bacterial sources such as *V. cholerae*[44] and *S. typhimurium*[45] catalyze the hydrolysis of sialic acids with retention of configuration at C-2 of the sialic acid residue, initially releasing the α-anomer.

1.4 Neuraminic acid glycals

The 2,3-unsaturated sialic acid, 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (Neu5Ac2en, DANA), has been found in free form in nature, and inhibits most exosialidases with a Ki of about 10^{-4} to 10^{-6} M[46],[47],[48]. It is postulated that Neu5Ac2en is a transition state analogue, mimicking the putative sialosyl cation intermediate in the proposed S_N1-like mechanism of sialidase catalysis (**Figure 1.5**).

Neu5Ac2en is structurally similar to the proposed transition state because it adopts a half-chair conformation with planarisation of the pyranose ring across the ring oxygen, C-2 and C-3 positions and there is no hydroxyl group on C-2. Neu5Ac2en has been widely used as a base template in studies aimed at developing more potent sialidase inhibitors.[47]

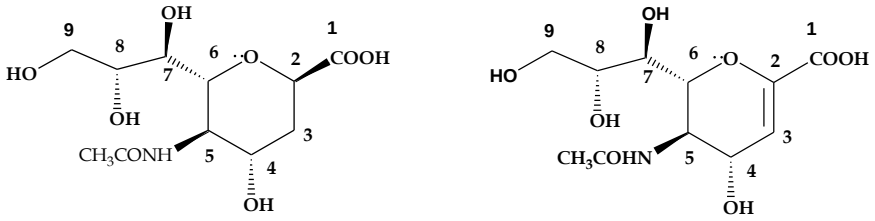


Figure 1.6: *First tested compunds as sialidase inhibitors*

At the beginning, an initial focus in the discovery of sialidase inhibitors was on substrate-like Neu5Ac derivatives, particularly 2-deoxy-α-D-*N*-acetylneuraminic acid (2-deoxy-α-Neu5Ac) derivatives (**Figure 1.6**)[47]. Based on the understanding of the enzyme's catalytic mechanism, it is supposed that such compound might not be rapidly metabolized and should be recognized by the enzyme as a result of the compound's substrate/product-like characteristics. This derivative is the first compound produced by the von Itzstein group to be evaluated *in vivo* in a mouse model of influenza infection by Glaxo researchers led by Charles Penn and Janet Cameron[49]. This compound showed weak, but measurable, effects in animals infected with influenza virus. Penn, Cameron and colleagues evaluated

Neu5Ac2en in their established mouse model under identical conditions to those used for 2-deoxy−α-Neu5Ac, and it demonstrated a good activity in the mouse model experiments. On the basis of these experiments, the choice of carbohydrate template changed. Concomitantly, further refinement of a number of sialidase crystal structures in complex with Neu5Ac and the confirmed *in vivo* inhibitor, Neu5Ac2en, are successfully completed[40]. This improvement in structural resolution enabled the commencement of a fully fledged structure-based drug design effort based on these X-ray crystal structures. Computational chemistry techniques are used to probe the active site of influenza virus sialidase in an attempt to design structurally modified Neu5Ac2en derivatives that might be more potent inhibitors. Moreover, these X-ray structural studies identify residues within the active site that are conserved and changed in sialidases across viruses and bactiria and provided an exciting opportunity for the development of selective inhibitors. Thanks to this studies, we now dispose of two commercially available neuraminidase inhibitors as antiflenzal drugs: zanamivir[50] and oseltamivir[51] (**Figure 1.7**).

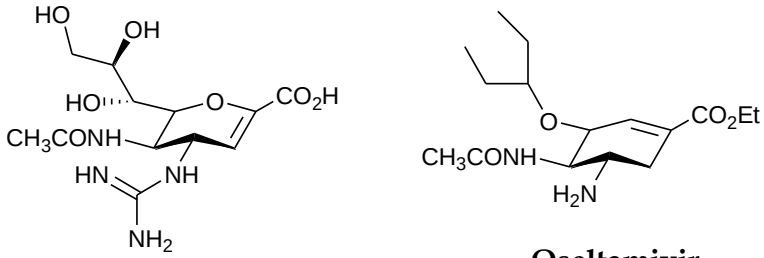


Figure 1.7: *Commercial antisialidase drugs as antiinfluenzals*

Zanamivir (4-deoxy-4-guanidino-Neu5Ac2en), designed and synthesised in the von Itzstein laboratories, developed by Glaxo Smith Kline and Biota, and sold under the tradename relenza, is administered as a nasal spray. The structure is based on Neu5Ac2en, with the natural C-4 hydroxyl group replaced by a guanidino substitutent. The design of zanamivir is achieved using the x-ray crystal structure of influenza virus A sialidase for detailed computational analysis of the enzyme's active site. Molecular modelling studies predict that

replacement of the C-4 hydroxyl group of Neu5Ac2en with an amino group would be energetically favourable. Replacement with a guanidino group is predicted to produce an inhibitor with even stronger binding properties through interaction with two conserved glutamic acid residues in the active site.

Oseltamivir is marketed by Roche and sold under the tradename of Tamiflu. The cyclohexene ring of oseltamivir sits in the same conformation as the dihydropyran ring of Neu5Ac2en, mimicking the proposed sialosyl cation transition-state structure. The amine substituent on the cyclohexene ring of oseltamivir is in the same position as the natural C-4 hydroxyl group of Neu5Ac2en, and the glycerol side chain of Neu5Ac2en has been replaced with a hydrophobic 3-pentyl ether. The result is a more lipophilic and orally bioavailable inhibitor.

These are the most successful examples of neuraminidases inhibitors, but, in these last years, many efforts have been spent to discover drug candidates against various kinds of viral, bacterial and mammalian sialidases.

1.5 Influenza A virus neuraminidase

Influenza A virus is a member of the orthomyxoviride family and has eight negative stranded RNA genomic segments within a lipid bilayer envelope. The envelope surface is spiked with multiple copies of hemagglutinins (HAs or Hs), NAs and fewer M2 ion-channel proteins. HAs and NAs are the antigens that define the particular strain of influenza. The variation of these proteins over time permits the virus to evade human immune response, thus necessitating the formulation of a new vaccine each year. The HAs are the sialic acid receptor-binding molecule that mediate the virus entry into the target cells, while the NAs cleave the glycosidic bound between the HAs of the new formed virions and the glycoconjugates of the host cell, spreading the infection (**Figure 1.8, A**). So, with

the inhibition of NAs the infection would be limited to one round of replication, not enough to cause the disease[52] (**Figure 1.8, B**).

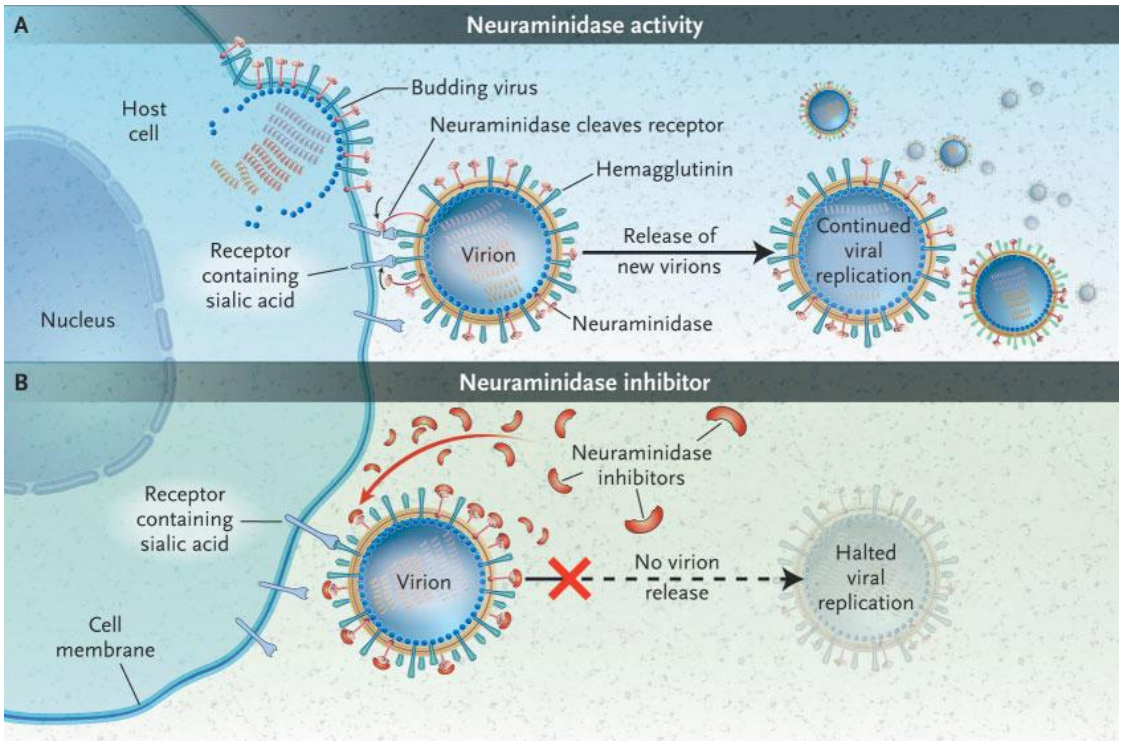


Figure 1.8: **A)** *Realising of new formed virion from the host cell through the neuraminidase activity;* **B)** *Action of neuraminidase inhibitors*

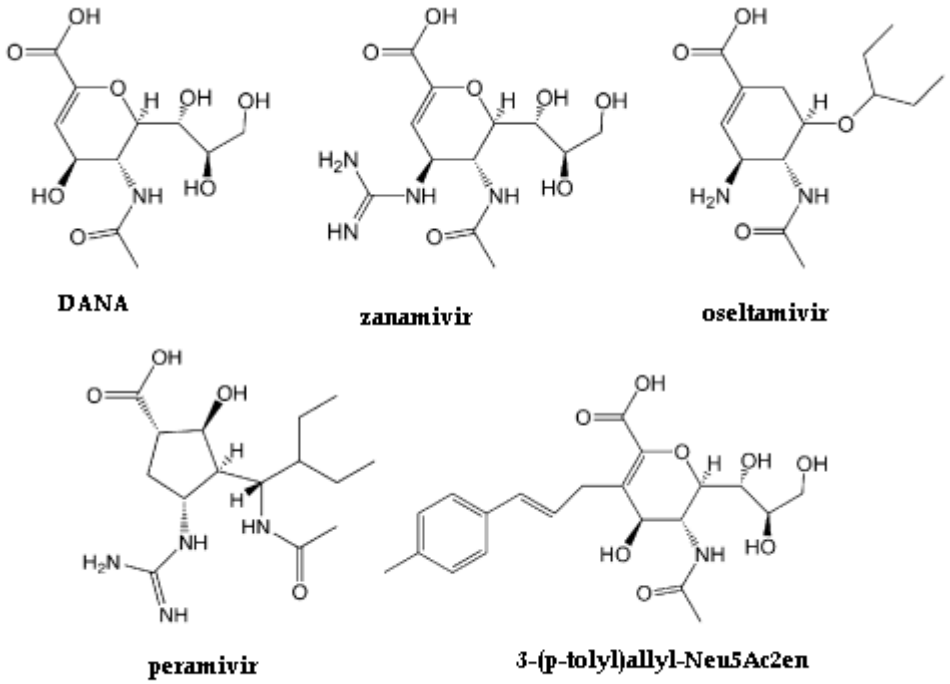


Figure 1.9: *Neuraminidase inhibitors against influenza virus*

The first NA inhibitoral compound discovered is the DANA (**Figure 1.9**), but it shows a low therapeutic impact on infected mice, primarily because of the rapid excretion. Crystal structure of NA in the early 1980s reveals a conserved sialic acid binding pocket. The X-ray structure of NA complexed with DANA bound in the active site[53],[21] shows that it binds with identical interactions as the natural substrate, sialic acid. Three arginine residues, 118, 292, and 371, bind the carboxylate **(Figure 1.10).** The oxygen and nitrogen atoms of the acetamido form hydrogen bonds with Arg 152 and a bound water molecule, respectively, whereas the methyl group lies in a hydrophobic pocket near Ile 222 and Trp 178. The O8- and O9-hydroxyl groups of the glycerol side chain are hydrogen bonded to Glu 276. The O4 hydroxyl sits at the entrance to a pocket formed, in part, by the acidic groups Glu 119, Asp 151, and Glu 227.

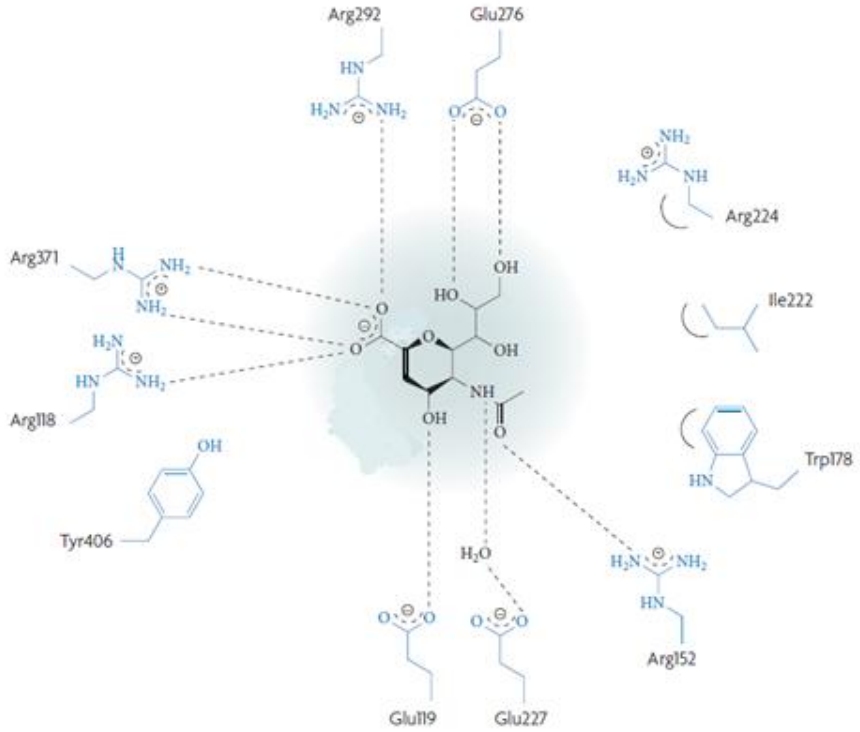


Figure 1.10: *Influenza virus neuraminidase active site*

Subsequently the structure and the structure-based modelling play an important role in the discovery of the potent substrate mimic zanamivir (commercially available inhalant drug) (**Figure 1.9**). This compound bears a guanidine group in place of the oxydrilic one at C-4 position. This modification improves the binding affinity with the enzyme thanks to the ionic interactions with the Glu 119, Asp 151, Glu 227.

The glycerol moiety of Neu5Ac derivatives has hydrogen interaction with Glu 276, and, attempts to replace by lipophilic group lead to the discovery of the oseltamivir (**Figure 1.9**), the second used drug against the influenza virus. In this case, the presence of the hydrophobic 1-ethylpropoxy group instead of the glycerol chain, of an amine group at C-4 position in place of the guanidine one, and the esterification of the carboxylate function reduce the compound polarity and increase the lipophilicity, obtaining an oral prodrug[54]. At the end, the peramivir[55] (**Figure 1.9**), approved as intramuscular injection for patients with acute viral infections, has a central ciclopentane ring in contrast of the cyclohexene ring of oseltamivir. Peramivir binding preserves the hydrophilic

interactions of the guanidine group (as Zanamivir), the hydrophobic interactions of the ethylpropoxy group (as Oseltamivir), the hydrogen bond of an OH group (as DANA) and the interactions of the carboxylate group (as all cited compounds).

The most used drug oseltamivir, with its ethylpropoxy group, has hydrophobic stacking with Glu 276 instead of the glycerol moiety bidentate hydrogen interactions with the same residue of Glu 276, whereas the ethylpropoxy group of oseltamivir. Influenza viruses have acquired the ability to discriminate oseltamivir from the substrate by developing NA mutations, that lead to develop oseltamivir resistance due to this different nature of the side chain interaction. On the other hand, the zanamivir, that resembles the substrate more, is less affected by resistance mutations compared to oseltamivir.

Furthermore, very recently, a transient ”150-cavity” adjacent to and accessible from the substrate-binding pocket has been discovered[56]. These structural results indicate that initial binding of sialic acid or analogue inhibitors requires the opening of the 150-loop which creates the 150-cavity, so modified DANA and zanamivir derivatives containing C-3 substitutions, as the recently synthesized 3-(p-tolyl)allyl-Neu5Ac2en[57] (**Figure 1.9**), could extend into the 150-cavity (**Figure 1.11**). Better assessment of this transient cavity and its evaluation for binding of small molecules may lead to new classes of NA-inhibiting influenza drugs.

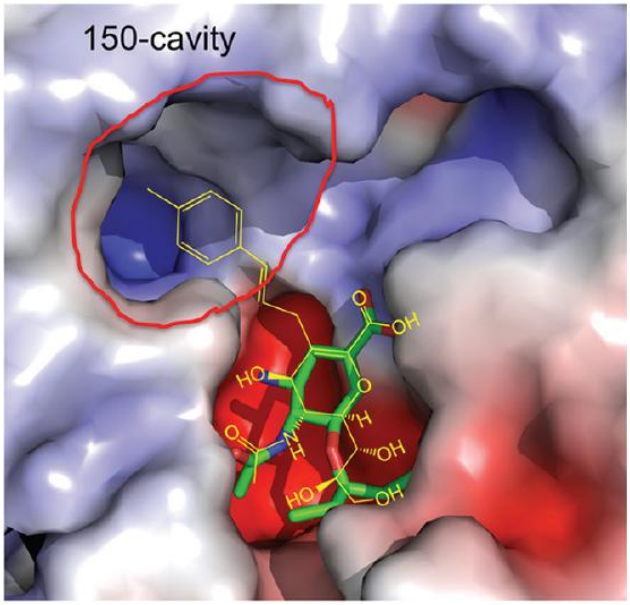


Figure 1.11: *3-(p-tolyl)allyl-Neu5Ac2en is modeled as a 2D structure (yellow) into the enzyme active site*

1.6 *Vibrio cholerae* neuraminidase

Cholera is a clinical-epidemiologic syndrome caused by *Vibrio cholerae*, usually of serogroup O1. In its severe form, cholera gravis, the clinical disease is characterized by the passage of voluminous stools of rice water character that rapidly lead to dehydration. Hypovolemic shock, acidosis, and death can ensue in adults, as well as in children, if prompt and appropriate treatment is not initiated[58]. *V. cholerae* neuraminidase (VCNA) plays a significant role in the pathogenesis of cholera: firstly, by reducing the viscosity of the gastrointestinal mucus, thereby facilitating the access of the bacterium to the target epithelial cells, and, mostly, by removing sialic acid from higher order gangliosides of the target cell membrane to unmask GM1, the receptor for cholera toxin (**Figure 1.12**)[34].

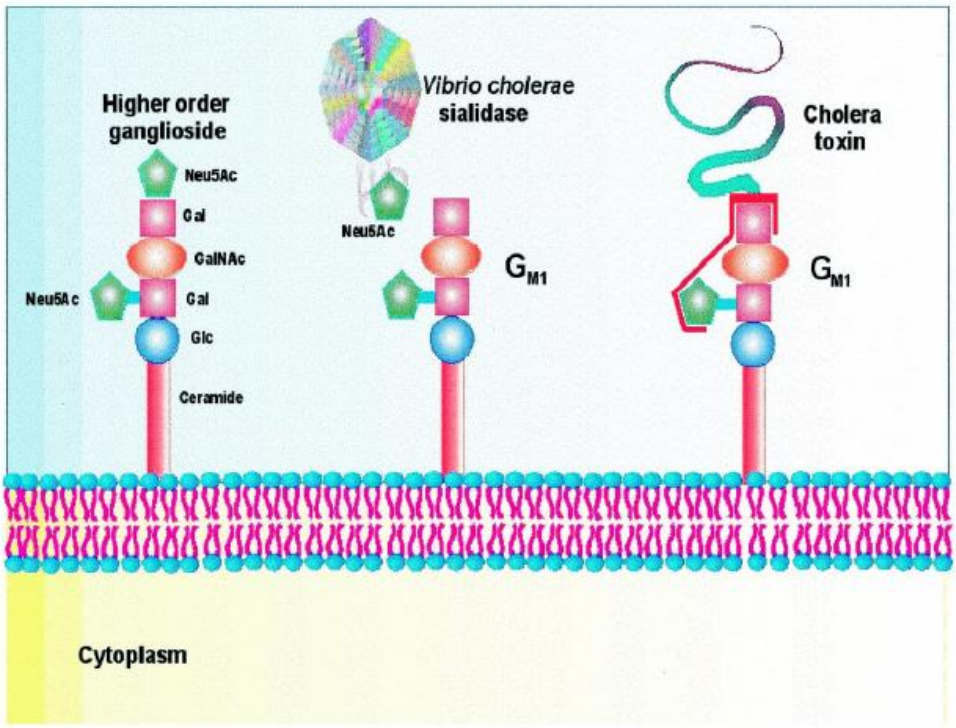


Figure 1.12: *Cleavage of a higher-order ganglioside by Vibrio cholerae sialidase to give GM1, the putative receptor for cholera toxin*

The 83 kDa enzyme folds into three distinct domains: the central catalytic domain that has the canonical neuraminidase β -propeller fold, very commun in bacterial sialidases, and is flanked by two domains which possess identical lectin-like topologies but without the metal-binding loops (**Figure 1.13**)[34].

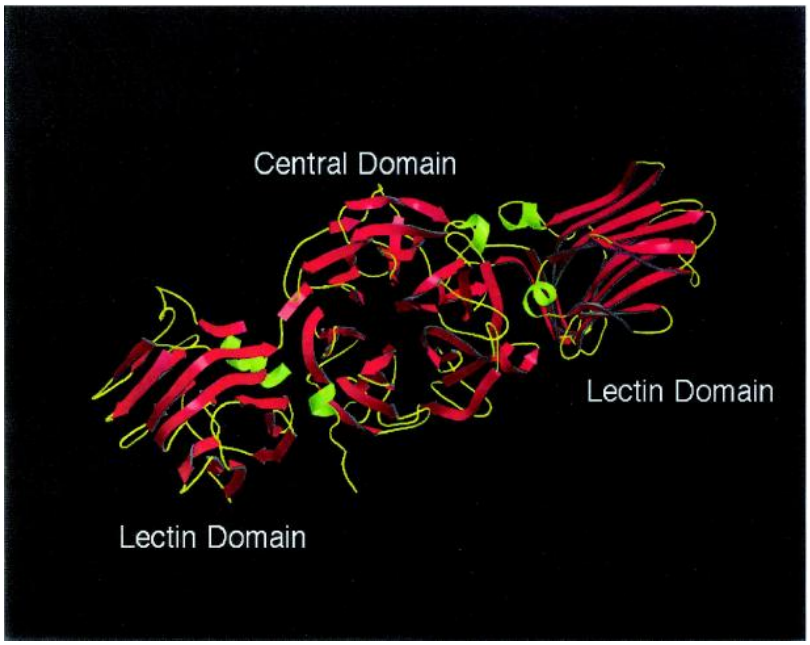
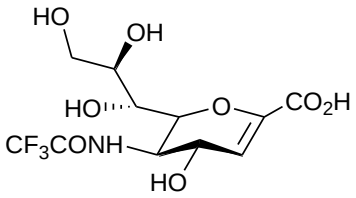


Figure 1.13: *Structure of Vibrio cholerae sialidase.*

The *V. cholerae* sialidase active site contains an array of residues that are conserved among several bacterial sialidases and different strains of influenza virus. The conserved array of residues in *V. cholerae* sialidase, detailed by Crennell et al.[34], is the one potentially involved in the enzyme catalytic mechanism. However, there are some differences between the sialidases in other areas of the active site, in particular in the vicinity of C-4 and of the C-6 glycerol side chain of Neu5Ac. The overall topology of active site cavity, in which DANA or Neu5Ac bind with the β-face of the pyranose ring towards the floor of the cavity, can be described as a pocket with a deep cavity directly below the C-4-epi position which extends underneath, around to the C-5 position. The upper surface of the pocket is formed by Asp 250 that sits directly above the C-4 position, Asn 318 which sits above the C-5 acetamido group, and hydrophobic residues Phe 638 and Leu 580 which encase the C-8/C-9 position. Furthermore the hydrophobic pocket of the C-5 acetamido substituent of Neu5Ac is relatively large, with quite extensive cavities adjacent to and below the C-5 acetamido group with a possible interaction between the active site residue Asn 318 and the *N*-acetamido group[59]. It is important to note that exchanging the acetamido group at C-5 with a trifluoroacetamido one, to have the *N*-trifluoroacetyl-

Neu5Ac2en (FANA) (**Figure 1.14**), we can obtain the most potent *V. Cholerae* NA inhibitor reported to date [60].



***N*-trifluoroacetyl-Neu5Ac2en (FANA)**

Figure 1.14: *The most potent neuraminidase Vibrio cholerae inhibitor: N-trifluoroacetyl-Neu5Ac2en*

The examination of the interactions of the hydroxyl group probe in the glycerol side chain binding region reveals that Asn 318 can interact with the C-7 hydroxyl group (3.1 Å) and C-9 hydroxyl group (2.5 Å). In addition, Asp 637 form a hydrogen bond with the C-8 hydroxyl group (2.9 Å).[59] The C-7 hydroxyl group could also participate in a hydrogen binding interaction with solvent exposed Asp 250 (2.5 Å). It is noteworthy that no favorable interaction involves the equatorial position at C-4 of Neu5Ac (**Figure 1.15**). Moreover, the analysis of the crystal structures of this bacterial sialidases shows that there is no cavity at C-4, like for the viral NAs, so justifying the inactivity of the influenza NA inhibitor zanamivir on this kind of NA[32].

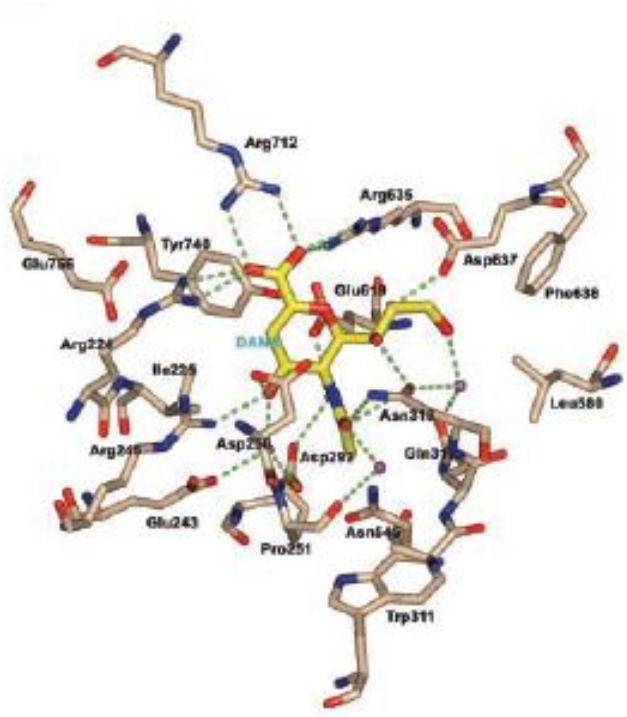


Figure 1.15: *Interaction between the V. Cholerae active site and DANA*

1.7 Newcastle disease virus (NDV) neuraminidase

Newcastle Disease Virus (NDV), a prototype of paramyxoviruses, is an avian enveloped RNA-negative strand virus that causes respiratory disease in domestic fowl, leading to huge economic losses in the poultry industry[61]. The envelope of NDV contains two associated glycoproteins that mediate viral entry: the haemagglutinin-neuraminidase (HN) and fusion (F) proteins. HN is the receptor-binding protein that recognizes and binds to sialoglycoconjugates at the cell surface and also has sialidase activity in separate sites[62]. Based on crystallographic studies of the HN of NDV, two sialic acid-binding site have been described[63]. Site I would support both receptor-destroying and receptor-binding activities, while site II only receptor binding activity. It remains unknown whether both sialic acid-binding sites might interact with the same cell surface molecule(s). Although it has long been known that NDV requires sialylated glycoconjugates for binding to cells, the exact nature of the receptor and the specific linkage for the sialic acid have not been well defined.

The interaction of the attachment protein (HN) with a cellular receptor is necessary for virus binding to target cells, and for the triggering of F protein-promoted fusion[63]. This fusion is considered to be driven by very large conformational changes following the triggering of the F protein, leading to exposure and insertion of the fusion peptide. The proteolytic processing of the nonfusogenic precursor forms (F_0) of paramyxovirus fusion proteins into the disulfide-linked heterodimer F_1+F_2 is essential for formation of fusogenically active proteins because it primes the protein for fusion by positioning the fusion peptide at the newly-formed N-terminus of F_1 (**Figure 1.16)[65].**

Figure 1.16: *NDV F protein*

Fusion-associated conformational changes in the F protein are considered to be irreversible, leading to a nonfusogenically active postfusion form of the protein. Substantial evidence suggests that, for most members of the family, fusion triggering involves specific interactions of the cleaved, metastable F protein with its homotypic attachment protein. Upon receptor binding, the attachment protein 'transmits' a signal to the F protein, potentially through conformational changes in the attachment protein and changes in the F protein–attachment protein interaction (**Figure 1.17**). The time and place where the fusion and attachment proteins interact is critical to understand the mechanism of fusion control, but the details of these interactions are still under investigation. One proposed model suggests that the initial interaction between the two glycoproteins occurs within the endoplasmic reticulum at the time of synthesis, potentially allowing the attachment protein to hold the F protein in its prefusion conformation until after receptor binding[65].

This conformational change of F protein mediates the membrane fusion process between the virus and the target cell, promoting the entrance of NDV into the cytoplasm.

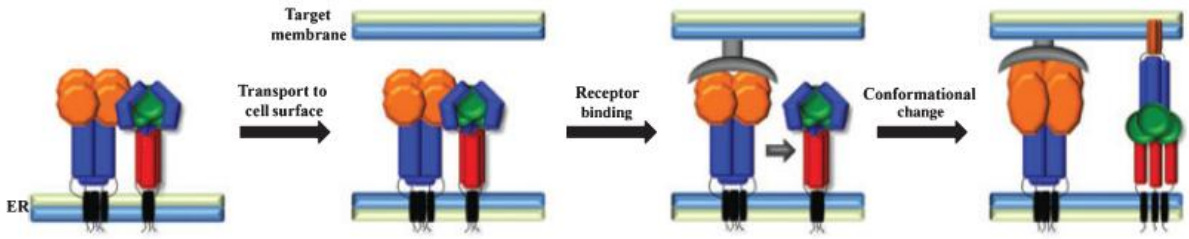


Figure 1.17: *NDV adhesion mechanism to the target cell (orange: NA; green and blue: F protein)*

This protein has a three-domain HN structure: a large hydrophilic carbohydrate-containing domain external to the viral membrane, a small hydrophobic domain spanning the membrane, and a small hydrophilic domain internal to the membrane[66]. The large hydrophilic domain contains the functional sites for cell attachment and neuraminidase activity, as well as the antigenic sites recognized by monoclonal antibodies (MAbs). It is not known whether HN molecule contains combined or separate active sites for hemagglutinin and neuraminidase activities. However, the preponderance of evidence suggests that cell-binding and neuraminidase activities occur at separate sites[67]. Most paramyxovirus HNs form disulfide-linked dimers. In Newcastle disease virus HN, a cysteine residue at 123 is identified to be responsible for the disulfide-linked dimer formation. In the complex soaked with DANA[48] the triad of arginines, Arg 163, Arg 405, and Arg 495, are positioned to interact with the sialic acid carboxylate. Beneath the ligand, Tyr 523 and Glu 390 hydrogen bond to each other, with Tyr 523 making additional potential hydrogen bonds to the ligand ring oxygen (O-6) and Arg 405. At the other end of the ligand, Asn 5 forms hydrogen bonds to a water molecule located underneath DANA, that is in a tight network with Ser 226, Tyr 306, and Glu 390. The glycerol moiety of DANA fits into a side pocket of the active site formed by Glu 247, Tyr 251, Tyr 306, Phe 353, Glu 390, and Arg 405. Glu 247 makes two hydrogen, bonds to O-7 and O-9 of DANA. O-8 of the glycerol moiety points down into the active site, forming a hydrogen bond with a buried water molecule involved in a net-work of interactions that includes the Glu 390 main

chain nitrogen, another buried water, the carbonyl of Gly 388, and the side chain of Asn 407. The Asn 544 carboxylate maintains a bidentate interaction with the Tyr 163 guanidinium group (**Figure 1.18**).

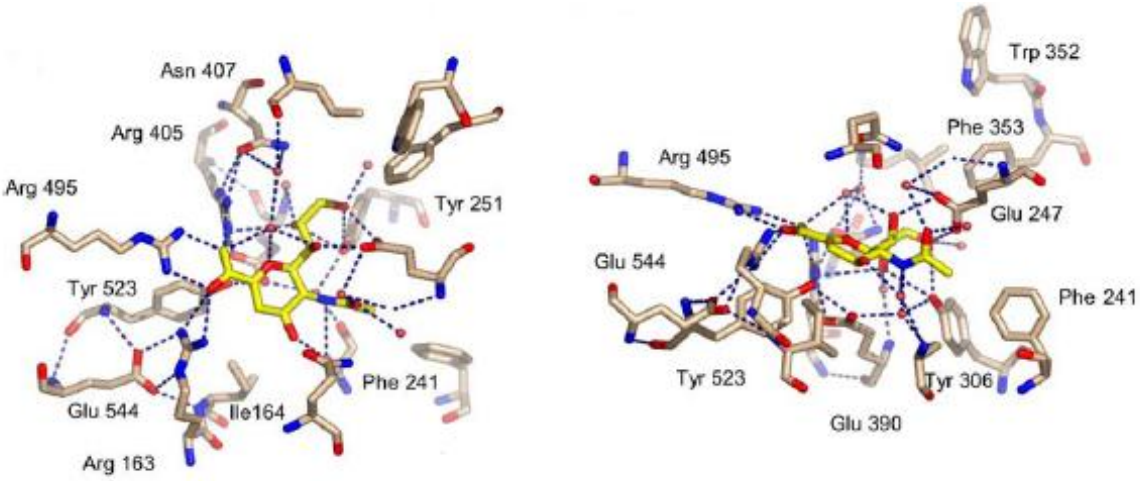


Figure 1.18: *Interactions between the NDV neuraminidase active site and DANA*

1.8 *Clostridium perfringens* neuraminidase

Clostridium perfringens is a Gram-positive anaerobic bacterium that causes life-threatening gas gangrene and enterotoxemia in humans. C. perfringens infections are characterized by the release of large amounts of toxins and enzymes that can cause massive destruction of the host tissue, putting the organism into the category of flesh-eating microbes[68].

The sialidases of *C. perfringens* have different cellular locations. NanH (43 kDa) lacks a signal peptide and is located in the cytoplasm. It has been proposed that NanH is involved in the cleavage of short oligosaccharides that enter the cell and are subsequently broken down for nutritional purposes[31]. By contrast, NanI (77 kDa) contains a signal peptide, is secreted from the cell, and is readily isolated from cell-free supernatants. A high-resolution structure of the catalytic domain of NanI in a complex with its sialic acid substrate has recently been described[46].

NanI may also play a role in nutrition, releasing sialic acid from higher-order gangliosides for subsequent transport into the cell. As a result of its location, NanI may also interact with the extracellular environment of the host tissue during infection, but neither sialidase was found to be essential for the pathogenesis of gas gangrene[31]. In the enzyme active site we can find the usual tri-arginal cluster (Arg 266, Arg 555, and Arg 615) that interacts with the carboxylate group of Neu5Ac. The position of the first arginine (Arg 266) is stabilized by a conserved glutamic acid (Glu 671). A tyrosine (Tyr 655) and a glutamic acid (Glu 539) hydrogen bond with each other and sit beneath and close to the C-1–C-2 bond of the substrate. A conserved feature of sialidase active sites is the acid/base catalyst, Asp 291. All sialidase active sites have a hydrophobic pocket to accommodate the *N*-acetyl group of the substrate, but the exact residues that form this pocket are generally not conserved. In the NanI structure, this pocket is made up of mostly aromatic side chains: Phe 347, Phe 353, Phe 460, Thr 345, Ile 327, and finally Trp 354 that forms a cap on the hydrophobic pocket (**Figure 1.19**). Of particular note is the appearance of seven water molecules in the covalent complex, compared with only two in the Neu5Ac complex. In the covalent complex, the relaxed conformation of the ring places the C-1 carboxylate group into the equatorial position, and this now hydrogen bonds to two of these waters. Several studies have indicated that water rearrangement plays an important role in carbohydrate-protein interactions. The ordering of five extra water molecules in the covalent complex would be expected to decrease the entropy, and subsequently increase the free energy of the system. This increase in free energy would serve to destabilize the covalent intermediate and may act to drive the reaction forward to product formation. Unique to NanI, compared with other sialidases, is Trp 354 acting as a lid on the hydrophobic pocket but extending over the glycerol group of sialic acid. It may shield this part of the active site from bulk solvent and help to contribute to the strength of the hydrogen bonds made by Gln 493 and Tyr 485 to the glycerol group through desolvation effects.

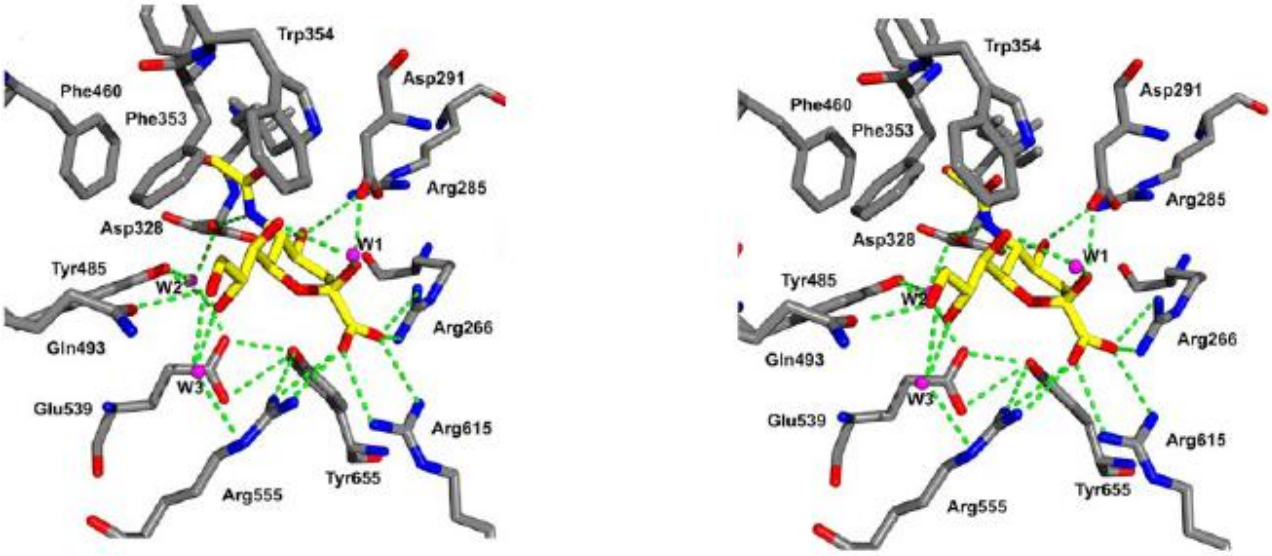


Figure 1.19: *Interactions between C. Perfigens neuraminidase and DANA*

1.9 Mammalian Sialidase, Neu 3

Sialidases of mammalian origin have been implicated in the modulation of various functional molecules involved in many biological processes, whereas the functions of their microbial orthologues appear only to be limited to nutrition and pathogenesis[69]. The four types of mammalian sialidases have been cloned and classified according to their major intracellular location. In human, they are designated intralysosomal sialidase (Neu1), cytosolic sialidase (Neu2), plasma membrane-associated sialidase (Neu3), and lysosomal or mitochondrial membrane-associated sialidase (Neu4). These sialidase isoforms (Neus) also differ in enzymatic properties including substrate specificities and immunological properties. In particular, Neu1 hardly hydrolyzes gangliosides and Neu3 acts preferentially on gangliosides but not glycoproteins. It is a characteristic feature of Neu4 that it acts on mucin[70]. With regard to the glycosidic linkage specificity, oligosaccharide substrates possessing the α-2,3 sialyl linkage are hydrolyzed faster than those with α-2,6 and α-2,8 linkages by Neu1[71] and Neu2[26], whereas Neu3 hydrolyzes gangliosides with α-2,3 (GM3) and α-2,8 (GD3) linkages almost equally and faster than those with α2-6 linkage (synthetic GM3)[72].

Aberrant expression of human sialidases has been found to be associated with the development of various pathological conditions[73]. Particularly, Neu3 is found to be significantly up-regulated in various human cancers and its upregulation is correlated with suppression of apoptosis and promotion of motility in cancer cells[3]. Inhibitory role of Neu3 in apoptosis is proposed to be mediated by accumulation of a possible sialidase product lactosyl ceramide (Lac-Cer), which induces Bcl2 expression or by rapid degradation of GD3. In addition, Neu3 mediated gangliosides depletion results in the activation of integrininduced kinase/Akt, followed by deactivation of caspase-9 in SCCl2 cells[74]. Moreover, polymorphism of the Neu3 gene in diabetic patients is discovered and found to be deeply involved in the onset of type II diabetes, in fact Neu3 is also found to be involved in insulin signaling in two ways[75]. First is the negative regulation of insulin signaling by associating with the Grb2 protein and second is the suppression of insulin receptor (IR) phosphorylation through the modulation of gangliosides. Taken together, these anomalous functions of Neu3 are different from the possible regulatory functions of other human sialidases. So selective Neu3 inhibition may be a useful approach in cancer and diabetes therapy or in any case would be a valuable tool for exploring differential functions of human sialidases.

Active site crevice of predicted enzymes is organized in the center of the β-propeller fold in the same fashion as in bacterial and viral neuramindases[72]. Notably, active site volume of Neu3 is smaller (216 $Å^3$) than active site of other human sialidases (277 $Å^3$ for Neu1, 227 $Å^3$ for Neu2 and 242 $Å^3$ 3 for Neu4), which might help for designing selective inhibitors. A common strategy for the design of sialidase inhibitors relies on the use of DANA as a transition state analog. The inhibition of Neu3 by DANA has been reported by several groups, and is usually found to be in the mid-micromolar range. Interestingly, zanamivir, a potent viral NA inhibitor has been reported to have low micromolar activity against Neu3, while oseltamivir is essentially inactive against the enzyme. Examination of the structures of DANA in the Neu3 active site suggests that there

is the arginine triad (Arg 25, Arg 245, Arg 340) that binds to 2-carboxylate and a tyrosine residue (Tyr 370), which stabilizes the sialyl–enzyme intermediate during catalysis, are highly conserved for all the sialidases, and one residue, His 277, is in addition, interacting with the 2-carboxylate group of DANA. There are four aminoacid residues (Asp 50, Asn 43, Arg 45, and Asn 88) that interact with 4-hydroxyl. The 5-*N*-acetyl coordinating residues that define the hydrophobic pocket are Asn 88, Tyr 179 and Asn 225. C-9 of the 6-glycerol moiety of Neu3 in crystal structure forms hydrophobic contact with Val 224 and His 227. Likewise O-7 forms hydrogen bonding with His 220 and Asn 264, O-8 forms hydrogen bonds with identical residues of Tyr 179, Asn 225 and Arg 245. Similarly O-9 forms hydrogen bonds with Asn 113, Tyr 179, Tyr 181 and His 277 (**Figure 1.19** and **Figure 1.20**).

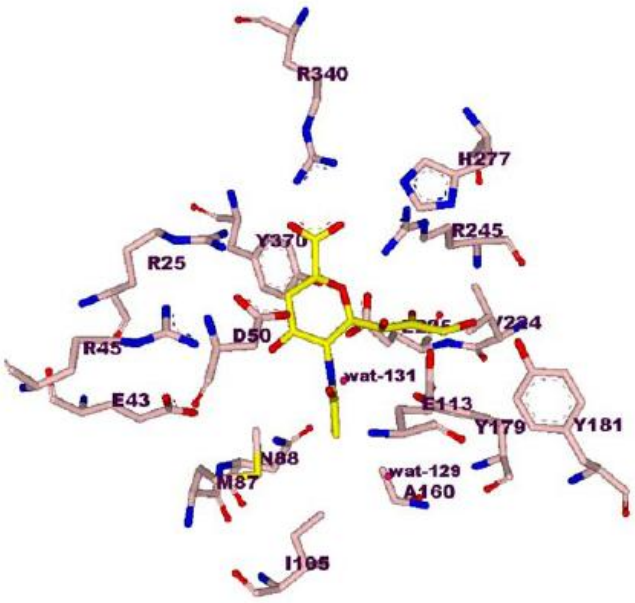


Figure 1.20: *DANA in the NEU 3 active site*

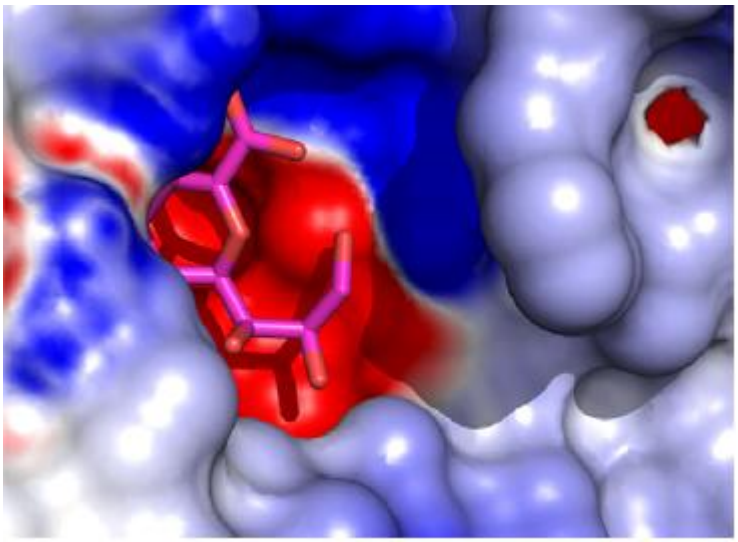


Figure 1.21*: DANA in the NEU 3 active site (3D imagine)*

2 AIM OF THE WORK

2. Aim of the work

This work represents a part of a larger project directed to research new pharmaceutical and biochemical tools related to sialidase activity and to expand the structure/activity relationship comprehension of sialidase inhibitors through the synthesis of new unsaturated sialic acid analogues and their biological evaluation.

In particular, the present work focuses on the development of simple procedures to rapidly modify positions 4 and 5 of DANA analogues (**Figure 2.1, A**), fundamental for the enzyme-substrate interaction; and on the synthesis of new 3,4-unsaturated Neu5Ac conjugates (**Figure 2.1, B**) possibly useful both as neuraminidase inhibitors and as synthetic intermediate to one pot functionalize monosaccharide positions 3 and 4 (**Figure 2.1, C**).

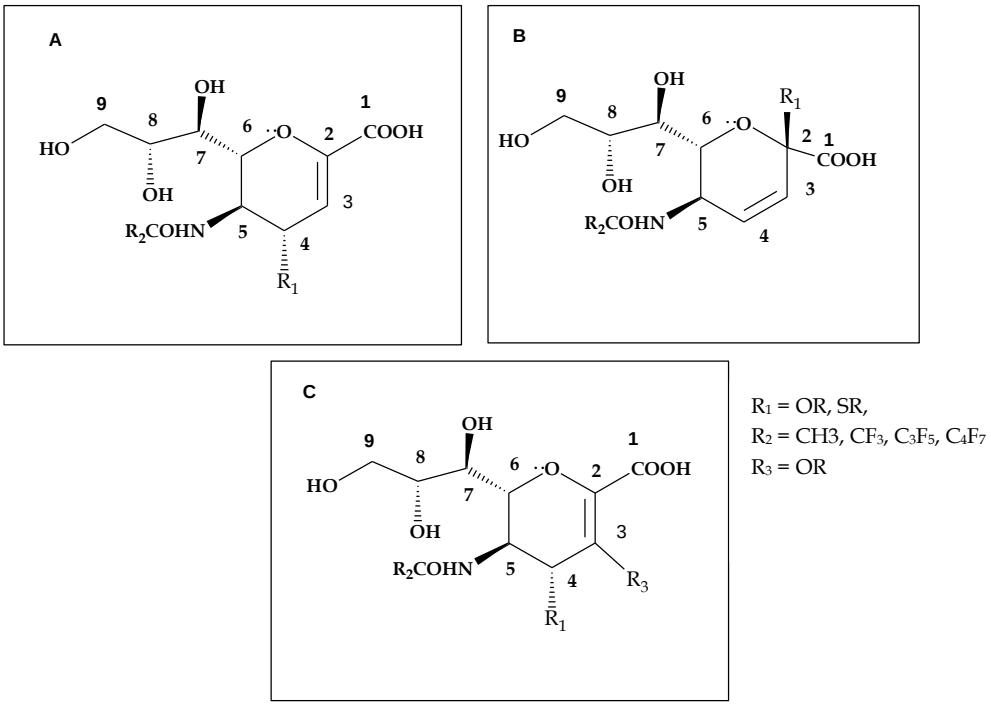
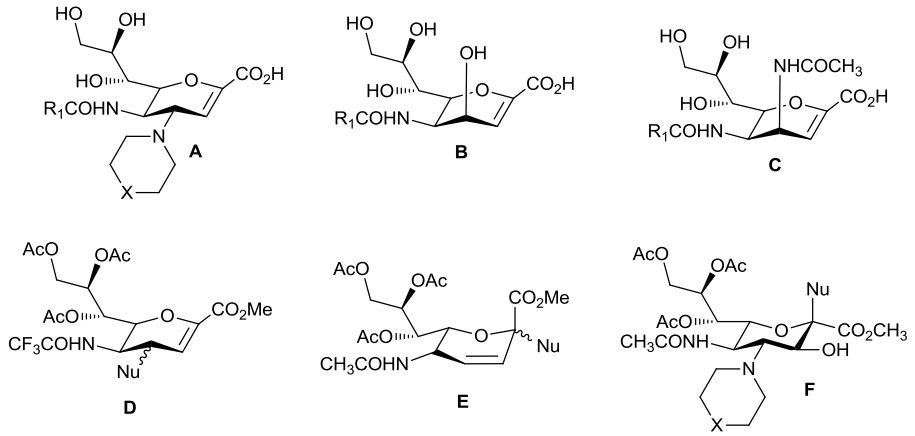


Figure 2.1: *General structure of the Neu5Ac derivatives we propose to synthesize (I): A, 4,5 modified DANA analogues; B, 3,4 unsaturated, 2modified DANA analogues; C, 3,4,5 modified DANA analogues.*

More in detail, at the beginning, we intend to set up a first rapid protocol to obtain C-4α cyclic amines (morpholine or piperidine) substituted glycals of 5-*N*-acetylated and 5-*N*-perfluoroacylated neuraminic acid (**Figure 2.2, A**); a second protocol to synthesize 4β-hydroxy DANA and FANA derivatives (**Figure 2.2, B**); a third protocol afford 4β-*N*-acylated Neu5Ac2en congeners (**Figure 2.2, C**).
Moreover, while we are accomplishing the previous protocols, we discover new findings that lead us to extend our initial objectives. In fact, we plan to set up also a versatile and wide applicable nucleophilic substitution on position 4 of DANA derivatives to afford an high number of compounds possibly useful as sialidase inhibitors (**Figure 2.2, D**).
Finally, we direct our attention to the little investigated 2-substituted 3,4-unsaturated analogues (**Figure 2.2, E**), with the aim to set up another innovative nucleophilic substitution, this time, orientated to the anomeric position 2 of the Neu5Ac ring. In fact, these compounds can be transition-state intermediate-like inhibitors of viral, bacterial, human sialidases too[76], but can also represent an useful tool to functionalize position C-3 of Neu5Ac, central for the interaction with influenza A virus neuraminidase binding site[56]. Furthermore we begin to explore the possibility to rapidly functionalize the 3,4 double bound to obtain new derivatives substituted at position 3, with an alcohol function, and at position 4, with a cyclic amine as shown in **Figure 2.2 F**.



R_1 = CH_3, CF_3, C_3F_5, C_4F_7; Nu = OR, SR, X, RSO_2NH; X = O, CH_2;

Figure 2.2: *General structures of the target compounds we propose to synthesize (II)*

To conclude, we program to test the inhibition activity of the free obtained compounds through preliminary biological activity assays on neuraminidases from *Vibrio cholerae*, NDV, *clostridium perfringens* and on mammalian sialidase Neu3, to, eventually find if there is any active compound to be used as biochemical tool to selectively inhibit a specific sialidase type.

3. RESULTS AND DISCUSSION

3. Results and discussion

In this section we will discuss in the order all the experimental results, starting from the description of the synthetic protocols we set up to obtain new unsaturated Neu5Ac derivatives, and clarifying also some particular chemical behavior of sialic acid glycals. Then we continue our discussion reporting the preliminary biological assays of some new obtained compounds on different kind of sialidases to evaluate their inhibition activity.

3.1 Synthesis of C-4α aminated derivatives of normal and *N*-perfluorinated Neu5Ac2en

This protocol is set up to synthesize the Neu5Ac2en derivatives **1a-d**, **2a-d** as shown in the **figure 3.1**[77]. As we can observe, these compounds bear a C-4α basic substituent (morpholine **1a-d** and piperidine **2a-d**) as zanamivir, and, in addition glycals **1b-d**, **2b-d** have a perfluorinated amide at position 5, feature that seems to improve the *in vitro* neuraminidase inhibitor activity[60],[78] against both influenza A virus and *Vibrio cholerae.*

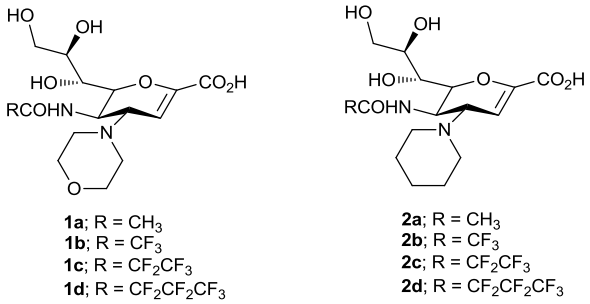
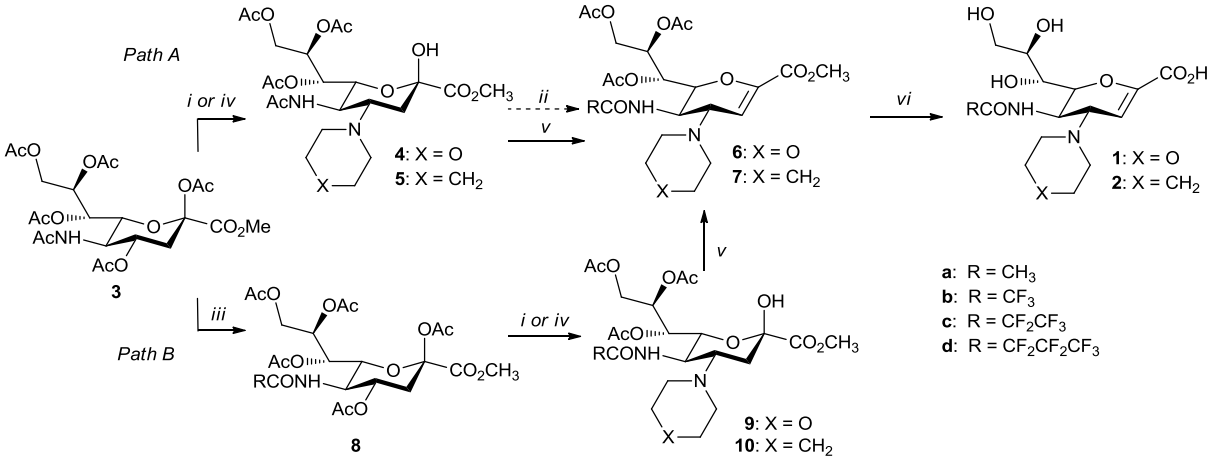


Figure 3.1: *Structure of compounds **1a-d**, **2a-d** we propose to synthesize*

When we start our work, an interesting protocol to easily insert a cyclic amine at C-4α position of Neu5Ac was reported in literature[79]. Thus, we think to apply this reaction as a first step of our hypothetical synthetic protocol (Scheme **3.1**, path A), starting from the protected Neu5Ac derivative **3** to obtain the aminated compounds **4** and **5**. Then, we plan to perform our "one pot" *N*-transacylation and unsaturation reaction[80],[81] to obtain protected *N*-perfluorinated glycals **6b-c** and **7b-c**. The final step is represented by the selectively hydrolysis of both the acetylic functions and the methyl ester of compounds **6b-c** and **7b-c** in presence of the labile fluorinated amides, to obtain the free glycals **1b-d**, **2b-d**.



Scheme 3.1: *Synthetic approaches for the synthesis of glycals* ***1a-d***, ***2a-d*** *(Path A and Path B). Reagents and conditions: i, morpholine or piperidine, Py, 25°C, 24 h; ii, TFAA or PFPAA or HFBAA, MeCN, 135 °C, 5–30 min; iii, TFAA or PFPAA or HFBAA, Et_3N, MeCN, 135 °C, 5 min; iv, morpholine or piperidine, CH_3CN, 60°C, 6-10 h; v, TFAA, TFA, MeCN, 135 °C, 5 min; vi, Et_3N-MeOH, 25 °C, 12 h.*

Therefore, following the path A (Scheme **3.1**), we treat the methyl ester of Neu5Ac **3** with morpholine or piperidine in pyridine at 25°C for 24h and we synthesize the precursors **4** and **5**, having respectively morpholine and piperidine as a substituent at the C-4 position and the free hydroxilic function at the C-2 position. Moreover, we improve the literature reported method by

exchanging the solvent (CH_3CN in place of pyridine), and increasing the temperature to 60 °C, so shortening the reaction time to 5 h, without affecting the yields.

Thus, we try to apply the direct *N*-transacylation reaction on these intermediates, in the same conditions previously reported by us in the absence of bases[81], to directly obtain the glycals **6b-c** and **7b-c**.

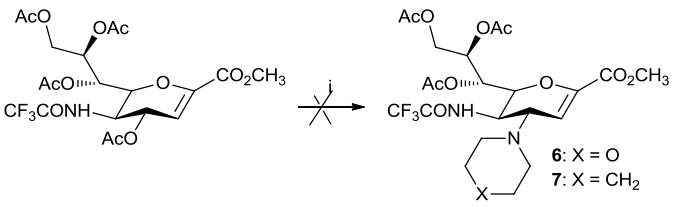
Unfortunately, this second step do not give the desired *N*-transacylate glycals in good yields, in fact, only the addition of 20 equivalents of perfluorinated anhydrides leads to the formation of the desired products although in low yields (20%). We do not observe any improvements of the *N*-transacylate glycal formation even when we perform the reaction on the substrates obtained by the riacetylation of the anomeric hydroxylic function. In fact, in all these cases, the main products achieved in good yields are glycals **6a** and **7a**, unsaturated but not *N*-transacylated. We think this unsatisfactory result is probably due to the C-4 cyclic amine hindrance that prevents the initial formation of the mixed imide occurring in *N*-transacylation reaction[80]. Anyway, we search for the best conditions for the dehydration of compounds **4** and **5**, performing different attempts varying the reaction conditions, as temperature or the acid catalyst. At the end, we find the acylation of the molecule 2-hydroxy group with TFAA, at 23 °C for 10 min, followed by treatment of the formed 2-trifluoroacetates with trifluoroacetic acid (TFA) in CH_3CN at 135 °C for 5 min can systematically lead to the desired glycals **6a** and **7a** in very good repetitive yields (80% and 78% respectively). The structure of both these glycals is assigned on the basis of their elemental analyses and physicochemical and spectroscopic properties.

All the collected NMR data show properties in agreement with the reported structures and, in particular, the coupling constant values between the olefinic proton at C-3 and the β-proton at C-4 signals, for both compounds ($J_{3,4}$ = 2.1 and 2.4 Hz) are diagnostic for the presence of an olefinic bond and of an allylic α-orientated amino group at C-4[82]. Thus, even if this route is very efficient to

synthesize the *N*-acetylated derivatives **6a** and **7a**, we have to find another synthetic protocol to achieve compounds **6b-d** and **7b-d**. Therefore, in order to obtain the C-4 aminated glycals having at C-5 a *N*-perfluorinated function, we try a second pathway (Scheme **3.1**, path B), reversing the reaction sequence. In fact, we decide to perform at first the *N*-transacylation reaction on the protected Neu5Ac **3**, and then the amination step, in order to prevent the chemical and steric interference of the basic substituent at C-4. Indeed, we are confidentent the reaction of direct amination at the C-4 position would proceed well also on the *N*-perfluorinate derivatives.

Consequently, as first step, we decide to synthesize the saturated *N*-transacylated compounds **8b-d**, using our *N*-transacylation reaction in the presence of Et_3N[83], since the basic medium of the reaction allows to retain the anomeric hydroxyl, essential for the successive direct C-4 amination[79]. At this point the introduction of morpholine or piperidine is attempted, first in the same reported conditions, using pyridine as solvent at room temperature for 24h, then with our modified procedure, employing CH_3CN and warming at 60°C to accelerate the reaction. Both the procedures lead at the derivatives **9b-d** and **10b-d** in good yields (ranging from 71% to 82%), thus demonstrating this reaction works well also on *N*-perfluorinate amides.

However, we have to underline that attempts performed on perfluorinate glycals, obtained by direct *N*-transacylation in the absence of base[81], gave no substitution at C-4 (Scheme **3.2**), supporting the proposed reaction mechanism[79].



Scheme 3.2 *Reagents and conditions: i, morpholine or piperidine, Py, 25°C, 24 h.*

The 2-hydroxy compounds **9b-d** and **10b-d** are then dehydrated, using the TFAA activation and the acid treatment, as described before, affording the corresponding glycals **6b-d** and **7b-d** in very satisfactory yields (ranging from 77% to 82%), with the correct physico-chemical properties.

At this point, to obtain the free glycals **1b-d** and **2b-d**, we have to solve the problem of the selective regeneration of the carboxylic and hydroxylic functions of the protected glycals **6b-d** and **7b-d**, retaining their perfluorinated amido groups. We try different basic reaction conditions on compounds **6b** and **7b**, but we obtain almost exclusively complex mixtures of compounds derived from a partial saponification of their labile trifluoracetic amides, difficult to be purified by flash chromatography.

Otherwise, good results are obtained on compounds **6a,c,d** and **7a,c,d**, having normal or heptafluorobutirric or pentafluoropropionic amides, using a solution of K_2CO_3 in MeOH. Attempts performed with aqueous solution of NH_3 in MeOH give good selective saponification of compounds **6b** and **7b**, but transform the sialic acid ester at C-1 into the corresponding amide. Finally, we obtain the free acidic glycals **1b** and **2b**, performing the hydrolysis with Et_3N, in aqueous methanol, at 23 °C for 12 h. The reaction allows to prepare the perfluoroacylated glycals **1b** and **2b** in good yields (93% and 85% respectively) and with the expected physicochemical properties. In particular the survival of the perfluorinated acyl groups is evident from the chemical shift of the carbonyl carbon in the ^{13}C-NMR spectra and from its coupling constants with the adjacent perfluorinated carbon, easily identifiable in the spectra.

3.2 Synthesis of C-4β hydroxy derivatives of normal and *N*-perfluorinated Neu5Ac2en

We set up a rapid protocol to synthesize C-4 epimers of normal and *N*-perfluorinated Neu5Ac2en derivatives **11a-d** (Figure **3.2**).

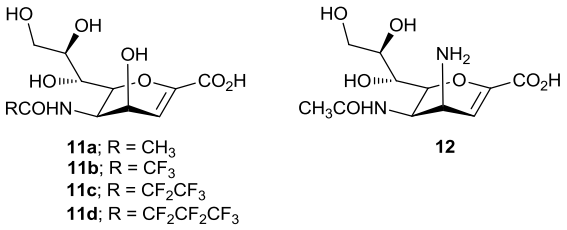
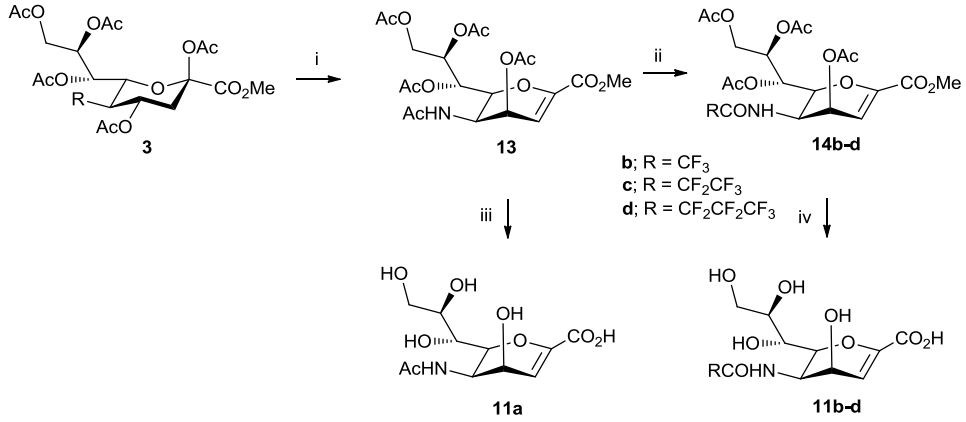


Figure 3.2: *Structure of target compounds **11a-d** we propose to synthesize; structure of the reported glycal **12***

We project to synthesize these compounds because we are interesting in the investigation of the C-4 substituent orientation influence in the enzyme/substrate interaction. In fact, these compounds are little considered in literature, despite it is reported that the 4-epi-amino-2,4-dideoxy-2,3-didehydro-*N*-acetylnneuramminic acid **12** (Figure **3.2**) displays an 10-fold greater inhibitor activity against influenza A virus neuraminidase than Neu5Ac2en probably for the interaction between the axial substituent at C-4 and a glutamate residue located just above the sugar ring in the active-site of the enzyme[41]. Furthermore, to the best of our knowledge, there are few methods to obtain C-4-epi congener of DANA **11a**[84],[85], but none to synthesize the perfluorinated analogue **11b**, despite the high reported antiviral activity of FANA[86].

In literature is reported that 4-epimer of DANA **11a** derived from the drastic acid treatment of Neu5Ac **3** and successive saponification of the esteric functions[84],[85]. So, we plan to start their synthesis from peracetylated Neu5Ac **3**, trating it with H_2SO_4/AcOH and Ac_2O for 48h to obtain **13**[85]. This

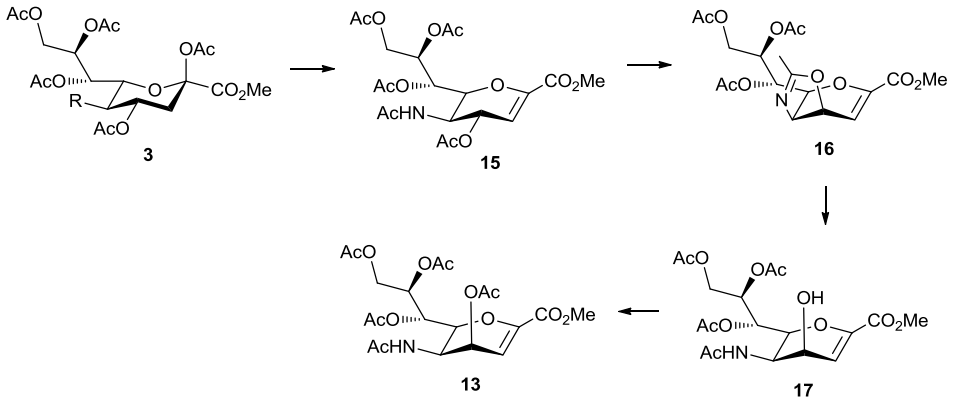
compound is then deprotected in classical basic conditions (K_2CO_3 in $MeOH/H_2O$) to obtain the known compound **11a**. Furthermore glycal **13** represents also the suitable precursor for the following *N*-transacylation reaction utilizing trifluoroacetic, pentafluoropropionic, heptafluorobutyrric anhydride (TFAA, PFPA, HFBA) to prepare glycals **14b-d.** The final selective saponification of their esteric functions can conduct to the desired products **11b-d** (Scheme **3.3**).



Scheme 3.3: *Synthetic approaches for the synthesis of glycals **11a-d**, (Path A and Path B). Reagents and conditions: i, H_2SO_4/AcOH/Ac_2O, 25°C, 48h; ii, TFAA or PFPAA or HFBAA, MeCN, 135 °C, 5–30 min; iii, K_2CO_3 in MeOHaq, 25°C, 2h; iv, Et_3N in MrOHaq, 25 °C, 12h.*

The simplest protocols reported in literature for the first step of our designed synthesis implicates the treatment of the peracetylated Neu5Ac **3** with Ac_2O, and H_2SO_4 or H_2SO_4/AcOH mixture for long reaction times (7 or 48 h)[84],[85]. Moreover, it worth nothing that we find some contrasting data about the epimeric ratio of the products[84],[87], indeed, in some reports, an isomeric mixture of α/β C-4 protected DANA derivatives results from this reaction. Thus, in order to set up a suitable protocol for our purpose, we intend to clarify the reaction mechanism. So, we monitor the reaction, performed by treating in acidic medium the protected Neu5Ac **3,** with TLC and ^{1}H-NMR analysis at different time and we ascertain the following synthetic progression (scheme **3.4**): β-elimination to protected DANA **15**, formation of 4,5-fused-

dihydro-oxazole **16** and its final hydrolysis during the work up to compound **17**. The successive reacetylation in presence of Ac_2O leads to the desired glycal **13**. Therefore, any traces of 4-α epimer **15** eventually present in the product mixture is due to the incomplete oxazoline ring derivative **16** formation.



Scheme 3.4*: Reaction progression to the formation of the C-4 epimer **13**. Reagents and conditions: H_2SO_4, Ac_2O in MeCN, 25°C 48h*

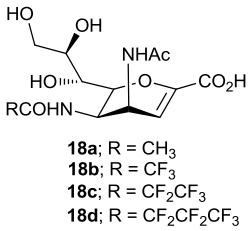
Once defined the course of the reaction, we reach the total conversion of starting material **3** into oxazoline derivative **16** performing a new protocol that requires to heat compound **3** at 80°C in a sealed tube for 5 minutes in presence of Ac_2O (10mmoleq) and H_2SO_4 (10mmoleq) in MeCN. Then, we also, search the best work up condition to obtain compound **13** in pure form. After different attempts, we found the best work up necessitates the addition of a stoichiometric amount of Et_3N and water. The successive extraction and the column chromatography purification leads to the glycal **13** in excellent yield (90%). This compound shows the same physical and spectroscopic characteristics of the one reported in literature, in particular the coupling constant value between the olefinic proton at C-3 and the α-proton at C-4 signals, ($J_{3,4}$ = 5.6 Hz) is diagnostic for the presence of a double bond and of an allylic β-orientated hydroxyl group at C-4[82].

Compound **13** is then rapidly saponificated using K_2CO_3 in MeOHaq to the reported free glycal **11a**.

Compound **13** is also used to perform our *N*-transacylation protocol at 135°C for 5 minutes in MeCN with TFAA, PFPAA and HFBAA and we obtain perfluorinated glycals **14b-d** in high yield (85-90%). Furthermore the same compounds **15b-d** can be rapidly achieved in comparable high yields (total yields 72-76%) simply subjecting the extracted mixture of precursor **13** to the just described transacylation protocol, without any further purifications. At the end, we accomplish the selective hydrolysis of ester functions in the presence of labile fluorinated amides, using our previously reported protocol, so we achieve the final products **11b-d** in very good yields (89-95%) by treatment the compounds **14b-d** with a MeOHaq solution of Et_3N for 12h.

3.3 Synthesis of C-4β *N*-acetamido derivatives of normal and *N*-perfluorinated Neu5Ac2en

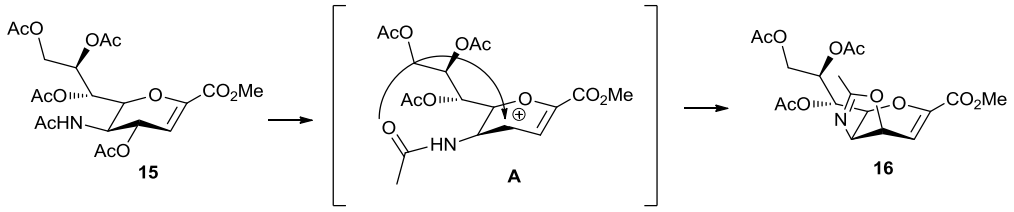
This paragraph is dedicated to the set up of the synthesis of glycals **18a-d** (Figure **3.3**) and is strictly related to the previous one.



18a; R = CH_3
18b; R = CF_3
18c; R = CF_2CF_3
18d; R = $CF_2CF_2CF_3$

Figure 3.3*:Structure of the compounds **18a-d***

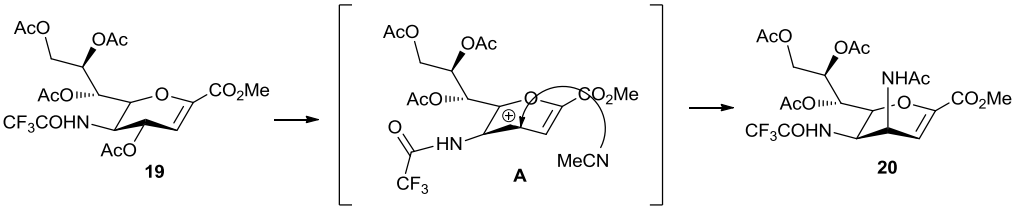
In fact, the initial purpose to synthesize these new products arises from the systematic study of the oxazoline **16** formation. During this study it results evident that the employ of acid catalyst promotes the elimination of the 4-acetyl group of the protected DANA **15** so generating an allylic carbocation **A** and, therefore, the oxazoline **16** derives from the intramolecular nucleophilic attack of the C-5 acetamido to adjacent C-4 allylic carbocation (Scheme **3.5**).



Scheme 3.5*: Oxazoline derivative **16** formation mechanism. Reagents and conditions: H_2SO_4, in MeCN, 50°C, 30 min.*

Te mechanism comprehension leads us to the synthesis design of the compounds **18a-d**, performing a driven Ritter reaction[88],[89] on the protected FANA **19**. This reaction is well known in the case of KDN[90],[91], the sialic acid congener bearing an hydroxy group at C-5 position; while as far as protected Neu5Ac concerned, the Ritter product is obtained in only two reports and just as byproduct of the oxazoline derivative **16** synthesis (< 10%)[92],[93].

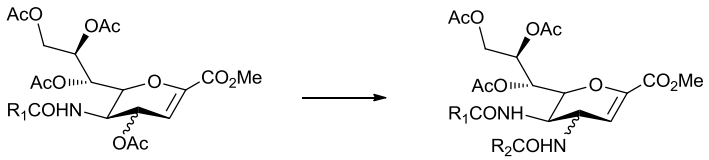
In our case, we are trusting we can disadvantage the oxazoline **16** reaction formation replacing the acetamide group with a trifluoroacetamide at position 5 (derivative **19**), so reducing the nucleophilic nature of its carbonyl, and, consequently, its propensity to intramolecularly attack the C-4 carbocation. Thus, starting from the protectected FANA **19**, we consider highly probable the intermolecular attack of MeCN, used as solvent, leading to the Ritter products **20** (Scheme **3.6**).



Scheme 3.6: *Ritter reaction on the protected FANA. Reagents and conditions: H_2SO_4, in MeCN, 50°C, 30 min.*

So, disposing of a simple and rapid method to synthesize *N*-perfluorinated glycals we synthesized compound **19**[81], and confident the electron withdrawing effect of the fluorine atoms is sufficient to permit the intermolecular insertion of the solvent, we perform the Ritter reaction on

compound **19**, in the same previously described conditions to obtain oxazoline **16** (H_2SO_4, at 80°C for 5 min in MeCN). According to our expectations, we obtain Ritter products **20** (4β-substituted), accompanied by minor amount of **21** (4α-substituted), in high yield and stereoselectivity (β:α; 9:1) (Table **3.1**, entry 1).



19 $R_1 = CF_3$ (4α)
14b $R_1 = CF_3$ (4β)
23 $R_1 = C_2F_5$ (4α)
24 $R_1 = C_3F_7$ (4α)
27 $R_1 = BnOCH_2$ (4α)

20 $R_1 = CF_3$; $R_2 = Me$ (4β)
21 $R_1 = CF_3$; $R_2 = Me$ (4α)
22 $R_1 = CF_3$; $R_2 = Et$ (4β)
25 $R_1 = C_2F_5$; $R_2 = Me$ (4β)
26 $R_1 = C_3F_7$; $R_2 = Me$ (4β)
28 $R_1 = BnOCH_2$; $R_2 = Me$ (4β)

Entry	Substrate	Catalyst	Conditions T/(°C) [t/min]	Product	Yields % (β/α ratio)
1	**19**	H_2SO_4	80 [5]	**20**+**21**	80 (9:1)
2	**19**	H_2SO_4	50 [30]	**20**	83 10:0)
3	**19**	TMSOTf	50 [3 h]	**20** + **21**	82 (2:1)
4	**19**	BF_3Et_2O	50 [3 h]	**20** + **21**	84 (2:1)
5	**19**	H_2SO_4	50 [30]	**22**	78 (10:0)
6	**14b**	H_2SO_4	50 [30]	**20**	78 (10:0)
7	**14b**	H_2SO_4	50 [30]	**22**	78 (10:0)
8	**23**	H_2SO_4	50 [30]	**25**	85 (10:0)
9	**24**	H_2SO_4	50 [30]	**26**	81 (10:0)
10	**27**	H_2SO_4	50 [30]	**28**	76 (10:0)

Table 3.1: *Preparation of Ritter glycal derivatives of sialic acids.*

Then we improve the stereoselectivity of the reaction, obtaining only β-epimer **20**, simply performing the reaction at lower temperature (50 °C) for 30 min (Table **3.1**, Entry 2). Successively, we evaluate the acidic catalyst influence replacing the H_2SO_4 with Lewis acids (BF_3Et_2O, or TMSOTf) (Table **3.1**, entries 3-4). As supposed, the only two isolated products are the Ritter reaction derivatives **20** and **21**, both in ratio 2:1. The observed variation of α/β selectivity is probably caused by to the different coordination effect of the acid

catalyst used with both the leaving group and the incoming nucleophile during the early stage of the reaction, as suggested in literature[94]. Then, we direct our attention to extend the utility and the applicability of this reaction replacing the acetonitrile with proprionitrile (Table **3.1**, entry 5) with H_2SO_4 at 50 °C for 30 min. Also in this case, we found the 4-β Ritter product **22**, without any detectable traces of the α epimer.

Finally, we verify that the reaction course is independent of the C-4 α or β configuration of the starting glycal, treating peracetylated FANA methyl ester **14b**, epimer at C-4 of glycal **19**, with both MeCN and EtCN (Table **3.1**, Entries 6 and 7). As expected, we obtain only the 4β-derivatives **20** and **22** respectively in high yields, as observed with the 4α-epimer **19**, so supporting our carbocation formation hypothesis.

We perform also the reaction on the pentafluoropropionic amide **23** and on the heptafluorobutiric amide **24**, both homologues of FANA, obtaining in these cases, the 4β-substituted glycals **25** and **26**, in comparable high yields and with no traces of the corresponding 4α-epimers (Table **3.1**, Entries 8 and 9).

Finally, achieved the Ritter products of Neu5Ac derivative and aware of the aforementioned literature on KDN congener, we devise to applied this protocol on the last congener of sialic acid family, protected Neu5Gc **27**. We are confident the mild electronactractive effect of its α-benzyloxyacetamido group can disadvantage the formation of the oxazoline respect to the solvent nucleophilic attack. In effect, the Ritter reaction is favored and affords the corresponding 4β-acetamido glycal **28**, in good yields and with complete β-diastereoselectivity (Table **3.1**, entry 10). This result completes the puzzle of the behavior of the glycals deriving from the three most representative members of sialic acid family, under Ritter reaction conditions. In fact, we can conclude this reaction, well know and widely utilized in the case of KDN derivatives, is applicable, without any artifice, also to Neu5Gc glycals. While in the case of Neu5Ac congeners, it is enough to use an appropriate *N*-perfluorinated derivative, easily available by direct *N*-transacylation[81].

At this point, we search for a possible rationalization of the observed β-product stereoselctivity, considering the steric effects involved in a direct attack at C-4. Thus, we perform a modeling study aimed to identify the preferred allylic carbocation intermediate conformation (Figure **3.4**). This research indicates the $^{5}H_{6}$ half chair conformer (Figure **3.4**, A), with a pseudoaxial chain at C-6, is more stable than the $^{6}H_{5}$ conformer (Figure **3.4**, B), with the same chain pseudoequatorial orientated [ΔE = 12 kJ mol^{-1}, in gas phase at the B3LYP/6-31G(d) level of calculation]. On the basis of these data, we consider highly probable the exclusive β-attack observed in the reaction can be due to the steric hindrance exercised by the chain at C-6 on the α-side of the $^{5}H_{6}$ half chair conformer.

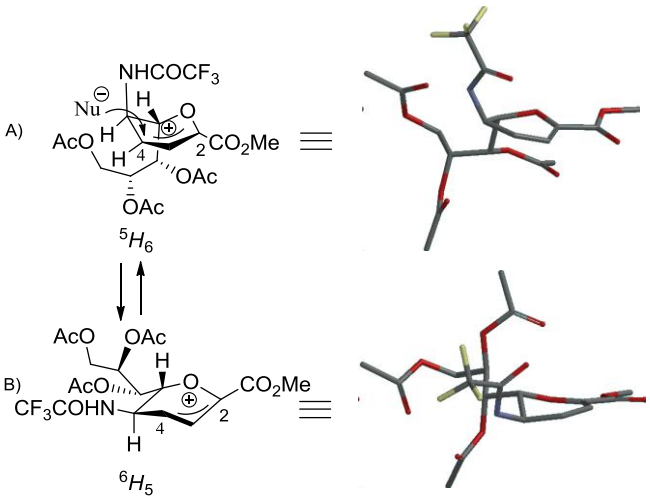
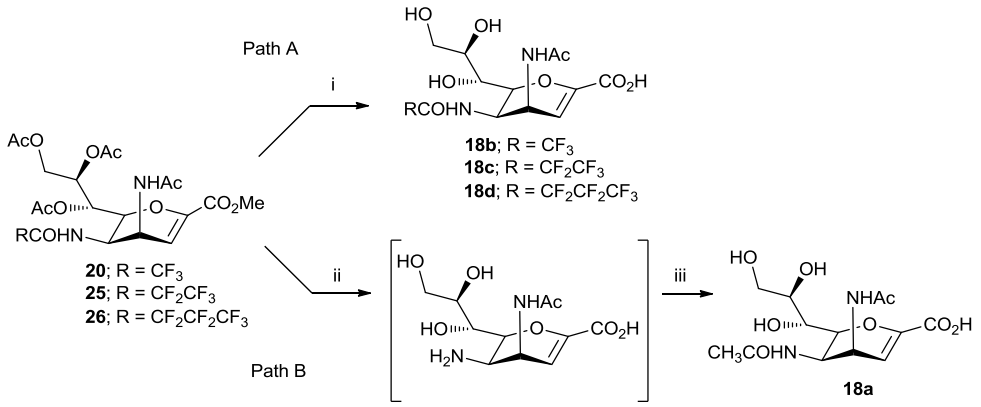


Figure 3.4: *Three-dimensional plots of the most-stable $^{5}H_{6}$ (A) and of the $^{6}H_{5}$ (B) conformers of allylic carbocation.*

Once found and clarified the Ritter reaction like applied to the sialic acid derivatives, we can easily obtain our C-4β *N*-acetamido glycals **18a-d**. In fact, with the simple above mentioned saponification with Et_3N in MeOHaq of compounds **20**, **25** and **26** we obtain the desired free glycals **18b-d** in high yields (Scheme **3.7**, path A). On the other hand, compound **18a** is easily synthesized by a simple hydrolysis of esteric and trifluoroacetamidic functions

of compound **20** with NaOH in MeOHaq, and selective reacylation of the aminic group at C-5 (90% yield) (Scheme **3.7**, path B).

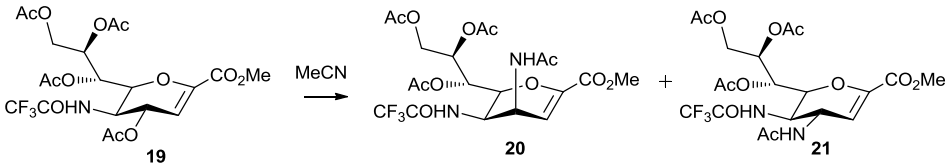


Scheme 3.7: *Final hydrolysis to obtain glycals 18b-d (Path a); to obtain glycal 18a (Path B).Reagents and conditions: i Et_3N-MeOH, 25 °C, 12h; ii, NaOH in MeOHaq, 25°C, 2h; iii Ac_2O in NaOH-MeOHaq, 25°C, 2h.*

3.4 General access to C-4 position of 2,3 unsaturated N-Acetylneuraminic acid *via* direct nucleophilic substitution

Encouraged by the Ritter like reaction preliminary results, we propose to amply and generalize the previously described procedure to easily access to the glycal position 4 with different kinds of nuclophiles.

In this section, I report our successful results on the study of the acid mediated allylic substitutions on DANA and FANA derivatives that permits to set up an extensive and straight protocol to afford an high number of potentially effective sialidase inhibitors. First of all, starting from our previously reported protocol, we intend to search for the best reaction conditions suitable for a general allylic substitution. So, we perform several experiments on protected FANA **19** using MeCN as nucleophile in stechiometric amounts varying different parameters (the solvent, type and quantity of the acid catalyst, and the temperature) (Table **3.2**).

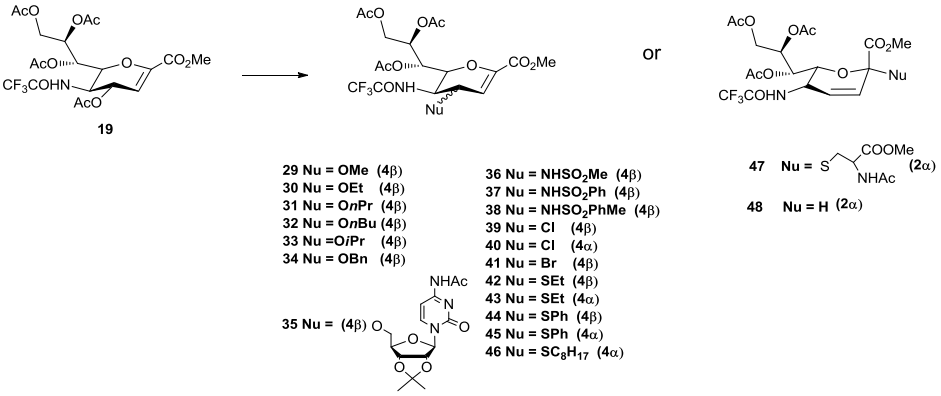


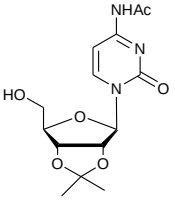
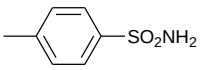
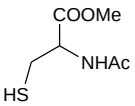
Entry	Catalyst	mMoleq	Conditions T/(°C) [t/h]	Solvent	Yields % (β/α ratio)
1	BF_3Et_2O	10	25 [48]	CH_2Cl_2	75 (3:2)
2	BF_3Et_2O	10	40 [1]	CH_2Cl_2	87 (3:2)
3	BF_3Et_2O	1	40 [48]	CH_2Cl_2	71 (3:2)
4	BF_3Et_2O	10	40 [1]	toluene	52 (3:2)
5	BF_3Et_2O	10	80 [1]	toluene	68 (3:2)
6	BF_3Et_2O	10	40 [1]	CH_3NO_2	81 (3:2)
7	BF_3Et_2O	10	40 [1]	DMF	27 (3:2)
8	BF_3Et_2O	10	80[1]	DMF	56 (3:2)
9	BF_3Et_2O	10	40 [1]	THF	33 (3:2)
10	TMSOTf	10	40 [1]	CH_2Cl_2	79 (3:2)

Table 3.2: *Set up general nucleophilic reaction conditions*

As summarized in Table **3.2**, the best conditions result performing the reaction in dichloromethane (DCM) as solvent, catalyzed by BF_3Et_2O (10 mMoleq) at 40°C for 1h (Table **3.2**, entry 2). The decrease of temperature (Table **3.2**, entry 1) or equivalents of catalyst (Table **3.2**, entry 3) leads to notably longer reaction time. While the solvent change (Table **3.2**, Entries 4,6,7 and 9), using toluene, DMF and THF instead of DCM, lowers the yields, not satisfactorily improved even at higher temperature (Table **3.2**, entries 5 and 8). Curiously, the epimeric *ratio* of the 4β-products **20** and of the 4α-product **21**, remains unvaried in all these experiments, also when catalyzed by TMSOTf (Table **3.2**, entry 10).

The most favorable conditions are extended to a great number of nucleophiles, reacting the protected FANA **19** with alcohols, sulfonamides, halides, hydride, thiols (Table **3.3**), to prove their applicability.



Entry	nucleophile	t (min)	Products	Yields % (β/α ratio)
1	MeOH	15	**29**	81 β only
2	EtOH	15	**30**	79 β only
3	*n*PrOH	30	**31**	71 β only
4	*n*BuOH	30	**32**	74 β only
5	*i*PrOH	60	**33**	75 (2:1)
6	BnOH	60	**34**	83 β only
7	Phenol	120	**no reaction**	0
8		120	**35**	58 β only
9	$MeSO_2NH_2$	15	**36**	87 β only
10	$PhSO_2NH_2$	15	**37**	88 β only
11		15	**38**	86 β only
12	TMSCl	15	**39**, **40**	78 (3:1)
13	TMSBr	30	**41**	73 β only
14	EtSH	15	**42**, **43**	85 (1:2)
15	PhSH	15	**44**, **45**	84 (1:5)
16	$C_8H_{17}SH$	30	**46**	61 α only
17		60	**47**	79 2-α only
18	TESH	15	**48**	86 2-α only

Experimental conditions: Glycal (0.2 mmol) in DCM (1.5 mL), Nuclephile (2 mmol), BF_3Et_2O (0.2 mmol), 40°C.

Table 3.3*: Nuclophilic substitution on protected FANA* ***19***

As observed, the reaction works well, and in high β-stereoselectivity on a great numbers of small (Table **3.3**, entries 1-4) and more hindered alcohols (Table **3.3**, entries 5 and 6). Unfortunately using phenol as nucleophile (Table **3.3**, Entry 7) the reaction fails, underlining the sensibility of our protocol to the decreased nucleophilic strength of aromatic alcohols. Interestingly we obtain satisfying results also using protected cytidine as nucleophile, affording to new nucleotide analogue **35** (Table **3.3**, entry 8).

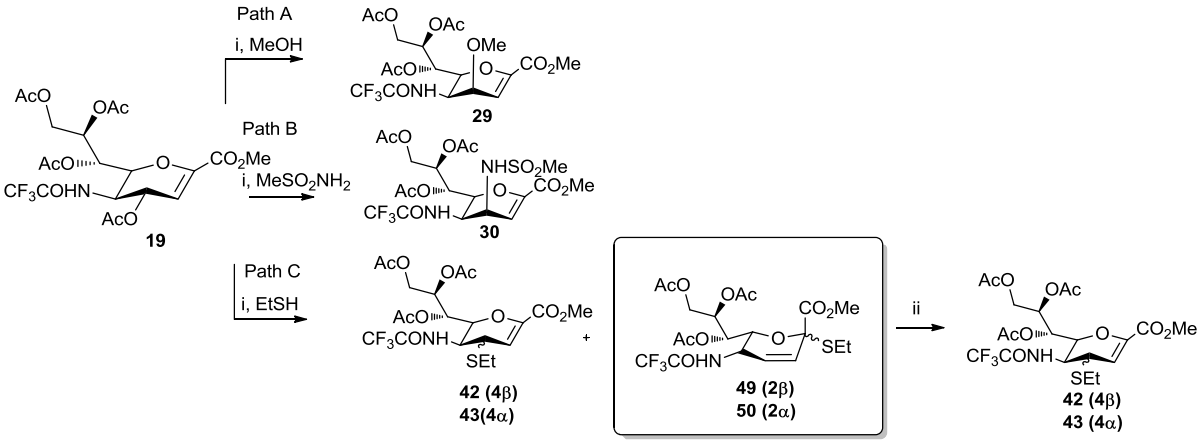
Similarly good results and dominant β-stereoselectivity are achieved also with sulphonamides (Table **3.3**, entries 9-11) and halides (Table **3.3**, entries 12 and 13).

On the contrary, reacting the protected FANA with thiols (Table **3.3**, entries 14-16), the 4-α epimers represent the mayor reaction products, with the α-steroselectivity that rises increasing the nucleophile size.

Moreover, surprisingly, in the reaction with *N*-acetyl cysteine methyl ester (Table **3.3**, entry 17), we obtain only the 2-α-thioglycoside 3,4 unsaturated isomer **47** in high yields (79%). Comparably, we afford the 2α-substituted 3,4 unsaturated isomer **48** rapidly and efficiently, using TESH as nucleophile (Table **3.3**, entry 18).

Thus, noting this *regio*- and *stero*- selectivity discrepancy, we hypotize as a possible explanation the likely involvement of the anomeric position 2. So we direct our attention to find any internal rearrangement from the glycal position 2 to the allylic position 4.

At first, we devise to perform the reactions on glycal **19** at less drastic conditions, decreasing the temperature and the mMoleq of the acid catalyst, using as nucleophile MeOH (Scheme **3.8**, Path A), $MeSO_2NH_2$ (Scheme **3.8**, Path B) and EtSH (Scheme **3.8**, Path C), and monitoring the reaction course through TLC and NMR analysis.

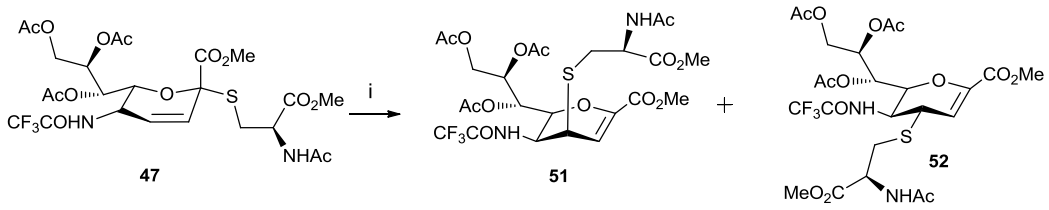


Scheme 3.8*: Allylic substitution and internal S-rearrangement. Reagents and conditions: i BF_3Et_2O (1 mMoleq), DCM, RT; ii, BF_3Et_2O (10 mMoleq), DCM, 40°C.*

As far as methanol and methanesulfonamide concerned, a sensible prolongation of reaction time is observed (from 15' to 14 h and 8 h respectively), and with several analyses during the reaction progression, just the final 4β- substituted epimers **29** and **36** are detected. On the other hand, the reaction with EtSH (Scheme **3.8**, Path C) at first shows a TLC spot that does not absorb in the UV-Vis range light having a slightly higher R_f respect to the starting glycal **19**. We extended the reaction until the complete starting material disappearance and we isolate the UV-vis and no UV-vis products present in the final reaction mixture. These products are the two 2-substituted isomers **49** and **50** (in 25% yields of inseparable isomeric mixture), together with the two 4-substituted analogues **42** and **43** (α/β 2:1, 55% total yields). The NMR spectra reveals the two 2-thioglycosides **49** and **50** are in isomeric *ratio* α/β 2:1.

To investigate their ability to rearrange, we heat at 40°C the anomeric mixture of **49** and **50** for 2 hours in dichloromethane, but we do not observe any significant change; so we add 10 Mmoleq of BF_3Et_2O and, in just 10 minutes, the 2-thioglicosides **49** and **50** are transformed into the two 4-substituted glycals **42** and **43** (isomeric *ratio* α/β 2:1) (Scheme **3.8**, Path C).

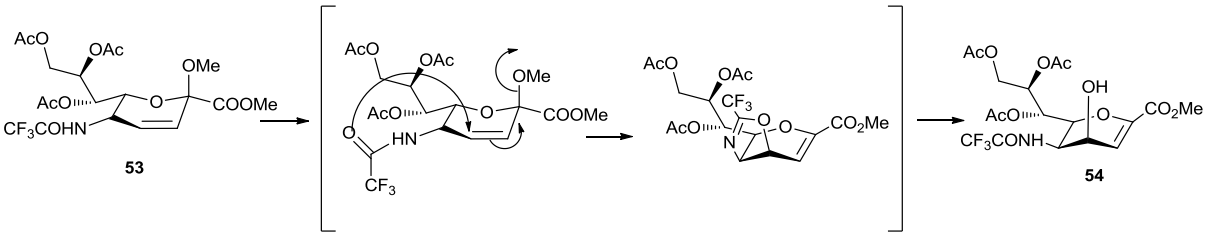
Similarly we perform the same rearrangement experiment using the 2-substituted cysteine 3,4 usaturated derivative **47** (Scheme **3.9**).



Scheme 3.9: *Internal rearrangement of cysteine nucleophile. Reagents and conditions: i, BF_3Et_2O (20 Mmoleq), DCM, 40°C, 2 h.*

This time we need 2 hours of heating and of 20 mMoleq of BF_3Et_2O, but we finally obtain the two 4-substituted isomers **51** and **52** in 2:1 (β:α) rate. These experiments demonstrate the attitude of sulfur nucleophiles, already reported for other unsaturated compounds,[95] to rearrange also in neuraminic acid structure, probably, at least in part, *via* carbocation intermediate.

Finally, to have a great support of our results we synthesize the *N*-perfluorinated 2-methyl glycoside **53** and we heat it in presence of BF_3Et_2O to exclude any oxygen possible internal rearrangement. According to our expectation the only detectable product is the 4β hydroxy epimer **54**, probably derived from the formation of an instable fluorinated oxazoline ring and its successive hydrolysis [96]. (Scheme **3.10**)



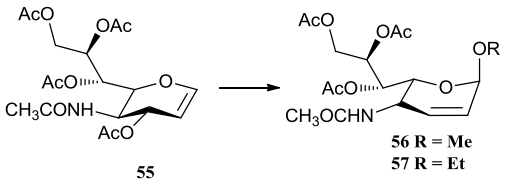
Scheme 3.10*: Formation of compound* **54** *from glycal* **53.** *Reagents and conditions: BF_3Et_2O in DCM, 40°C*

These experiments strongly support our thesis of the direct C-4 attack of alcohols and sulfonamides.

The preferred β-orientation of the direct C-4 attack, in this case, is likely caused by the most stable intermediate reaction conformer, as we previously discuss, that has the glycerol chain pseudoaxially orientated below the molecular plane. On the basis of these data, we considered highly probable the predominant β-attack observed in the reaction could be due to the steric hindrance exercised by the chain at C-6 on the α-side of this half chair conformer.

On the other hands thiols preferentially attack C-2 position, probably because of their marked nucleophile nature difference, but, in a second step, they can rearrange to the most stable allylic C-4 position, however, at the moment, any further assumption is highly speculative.

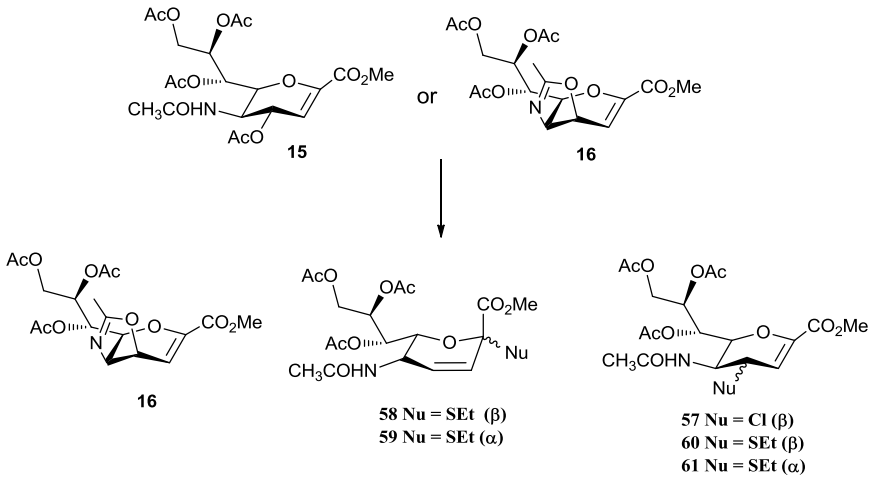
To complete these results, we wonder if the impediment to the C-2 position substitution, with consequent double bond shift to position 3,4, commonly reported in glycals of other neutral sugars[97], is represented by the extended conjugation system between the C-1 corboxylic group and the 2,3 double bond. In response of this question, we synthesize the decarboxylate DANA congener **55**, and perform our reaction with MeOH and EtOH as nucleophiles on this compound. In both cases we obtain the 2-substituted glycosides 3,4-unsaturated **56** and **57** in good yields (76% and 73% respectively) and total stereoselectivity (Scheme **3.11**).



Scheme 3.11*: Nucleophilic substitution on decarboxylated glycal. Reagents and conditions: ROH, BF_3Et_2O in DCM, 40°C,30min*

These experiments strongly support our hypothesis, so with alcohols and sulfonamides, the direct attack at C-4 is probably due to maintain the extended conjugation system between the carboxylic function at C1 and the 2,3 double bound, not present in the other neutral sugars considered in literature.

Moreover, we investigate with parallel experiments, the protected DANA **15** and the oxazoline derivative **16** propensity to undergo a wide nucleophilic substitution reaction (Table **3.4**).



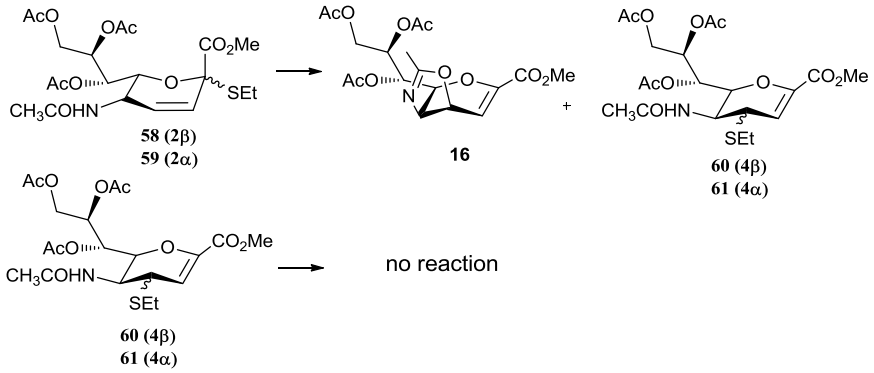
Entry	Sbstrate	nucleophile	Products	Total Yields %
1	**15**	MeOH	**16**	91
2	**16**	MeOH	**No reaction**	/
3	**15**	$MeSO_2NH_2$	**16**	90
4	**16**	$MeSO_2NH_2$	**No reaction**	/
5	**15**	TESH	**16**	93
6	**16**	TESH	**No reaction**	/
7	**15**	TMSCl	**57**	84
8	**16**	TMSCl	**57**	88
9	**15**	EtSH	**16,58,59,60,61**	89
10	**16**	EtSH	**58, 59**	93

Experimental conditions: Glycal (0.2 mmol) in DCM (1.5 mL), Nuclephile (2 mmol), BF_3Et_2O (0.2 mmol), 40°C.

Table 3.4*: Nucleophilic substitution on protected DANA **15** and oxazoline derivative **16***

As expected, performing the reaction on the glycal **15** with MeOH (Table **3.5**, Entry 1), $MeSO_2NH_2$ (Table **3.4**, Entry 3), and TESH (Table **3.4**, entry 5), we

obtain the oxazoline derivative **16** as the mayor product. Accordingly with these results, starting from the oxazoline derivative and performing the same reactions (Table **3.5**, entries 2,4 and 6) we recover the unchanged oxazoline. We obtain the 4-β product **57** performing the reaction with TMSCl both from compounds **15** and **16** in good yields (Table **3.4**, entries 7 and 8), as already reported by von Itzstein at al. with a similar procedure[98]. Interestingly, using EtSH as nucleophile and performing the reaction on the protecting DANA **15** we afford to the two C-2 anomers **58** and **59** (this time separable by simple flash chromatography), and the two C-4 epimers **60** and **61** (Table **3.4**, entry 9) together with the oxazoline **16**. However, performing the same reaction on the oxazoline **16** (Table **3.4**, entry 10), just the two epimers at position 2 (**58** and **59**) are isolated, as if the oxazoline, in this case, can prevent the access to the C-4 position. Also this time, we try to transpose the C-2 substituted compounds **58** and **59** to the C-4 position by simple heating in dichloromethane for two hours, but we do not observe any change in the reaction mixture, so we add BF_3Et_2O (Scheme **3.12**).



Scheme 3.12*: Ethathiol rearrangement study on Neu5Ac glycals. Reagents and conditions: BF_3Et_2O in DCM,40°C*

This addition leads to a rapid transformation of the thioglycosides **58** and **59** into the oxazoline derivative **16** (75% yields), accompanied by a small amount of the 4-substituted glycals **60** and **61** (17% in total yields). So the carbonyl group at C-5 position, another time, attacks the C-4, and, consequently, the double bond shits from 3,4 to 2,3 positions with ethanthiol elimination.

Anyway, it worth nothing that the C-4 *S*-substituted glycals are the final reaction products, in fact hating compounds **60** and **61** in the presence of BF_3Et_2O no modification is observed (Scheme 6).

It seems evident that oxazoline derivative **16** reaction formation is prevalent in these experiments precluding any general C-4 allylic substitution mediated by acid, so inducing on the FANA pathway to set up a straight and wide-applicable nucleophilic substitution at C-4 position.

In the end, these studies result in several new findings: first of all we provide an easy method to efficiently and rapidly functionalize C-4 position of Neu5Ac glycal analogues. Moreover, these new compounds, bearing a labile group at C-5 position can represent the suitable precursors of a new class of C-4, C-5 modified glycal, obtainable by a mild hydrolysis and selective reacylation of the amino group at C-5. Thus, we open the doors to the simple synthesis of a great number of compounds, potentially useful as sialidase inhibitors. Furthermore, we deeply study different aspects of our reaction, to explain the region- and the stereo- selectivity. We search and prove the internal rearrangement of thiol nucleophiles from position 2 to the more stable 4-isomer, with consequent double bond shift. While, with parallel research, we demonstrate the direct attack to position 4 of alcohol and sulfonamide nucleophiles, probably to maintain the extended conjugation system of the molecule. The study is extended also to DANA and oxazoline derivative, with a careful attention for their behavior when subjected to our protocol, justifying the employment of FANA analogue route. In this work, not just the successful procedure to access to C-4 position represents the fulfillment of a very relevant goal, but also the research to rationalize our data discloses important features of neuraminic acid glycal relevant for the synthesis of new sialidase inhibitors.

3.5 General protocol to synthesize new 3,4 unsaturated *N*-acetyl neuraminic acid conjugates and their possible derivatizations

The research of efficient synthetic methods for the preparation of *N*-acetylneuraminic acid (Neu5Ac) conjugates and modified structures has been a primary focus in carbohydrate chemistry, to their significant roles in physiological and pathological processes[99]. In particular, a great interest has been directed to the 2,3 unsaturated Neu5Ac derivatives, as proven sialidases inhibitor activity against viruses and bacteria[100], while, in contrast, very few reports are dedicated to investigate the chemistry and the therapeutical applicability of the 3,4-unsaturated analogues[76],[101].

Moreover, these compounds can be transition-state intermediate-like inhibitors of viral, bacterial, human sialidases, but can also represent an useful tool to funzionalize position C-3 of Neu5Ac, central for the interaction with influenza A virus neuraminidase binding site[56]. Thus, in this paragraph, we report our preliminary results on the successful development of a simple protocol to synthesized 3,4-unsaturated Neu5Ac derivatives, *via* direct and stereo-controlled nuclephilic substitution at C-2 (**A**, Figure **3.5**) and their possible derivatization as 3,4 dibromide (**B**, Figure **3.5**) or epoxide (**C**, Figure **3.5**) derivatives and successive nuclephilic attack to 4-aminated, 3-hydroxilated Neu5Ac analogues (**D**, Figure **3.5**).

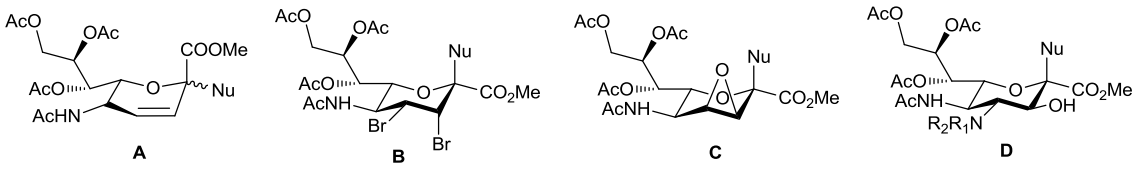
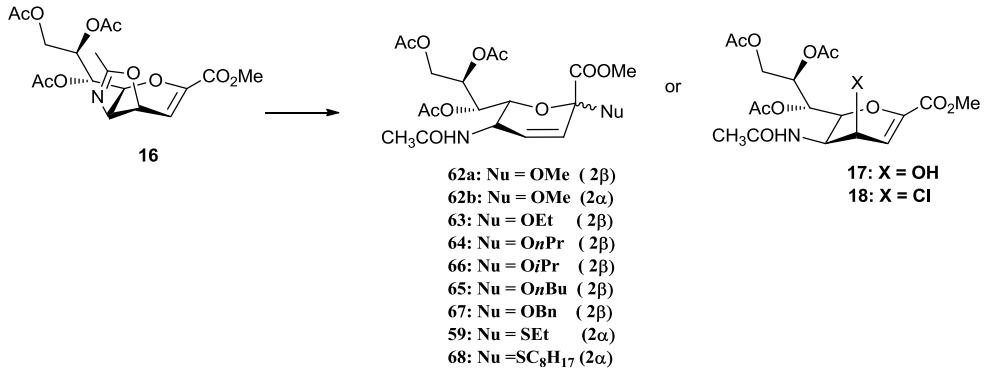


Figure 3.5*: General structures of the target compounds*

We first investigate the previously reported protocol by Ikeda et al.[76] to synthesize alchyl ketosides of 3,4 unsaturated Neu5Ac **A** starting from the 4,5-oxazoline derivative **16** and promoted by catalytic amount of montmorillonite K-10/$Bi(OTf)_3$. As reported, we obtain the desired β-methyl and ethyl

ketosides in good yields (Table **3.5**, entries 1, 2) and we observe a strong product recovery decrement using longer chain alcohols as nucleophiles (Table **3.5**, entries 3,4). For these reasons we devise to in depth study this reaction in order to extend its applicability. Thus, at the beginning, we compare the synergic effect of the two catalyst mixture with the single contribution of $Bi(OTf)_3$ and Montmorillonite K-10. Interestingly the reaction performed with $Bi(OTf)_3$ (Table 3.6, entry **5**) affords comparable yields (84%) of the 2-methilketosides but in different α/β *ratio*, while the reaction catalyzed by Montmorillonite do not occur (Table **3.5**, entry **6**).



Entry	Catalyst	Nuclueophile	Conditions T/(°C) [t/h]	Solvent	Products	Yields % (β/α ratio)
1	$Bi(OTf)_3$/Mont	MeOH	25 [48]	MeCN	**62a**	92 (β only)
2	$Bi(OTf)_3$/Mont	EtOH	25 [48]	MeCN	**63**	84 (β only)
3	$Bi(OTf)_3$/Mont	*n*PrOH	25 [48]	MeCN	**64**	61 (β only)
4	$Bi(OTf)_3$/Mont	*n*BuOH	25 [48]	MeCN	**65**	22 (β only)
5	$Bi(OTf)_3$	MeOH	25 [48]	MeCN	**62a**, **62b**	84 (1:1)
6	Mont K10	MeOH	25 [48]	MeCN	no reaction	-
7	BF_3Et_2O	MeOH	25 [48]	MeCN	**17**	35
8	TMSOTf	MeOH	25 [48]	MeCN	**17**	42
9	$FeCl_3$	MeOH	25 [48]	MeCN	**57**	83
10	$AlCl_3$	MeOH	25 [48]	MeCN	**57**	79
11	Al/mont K10	MeOH	25 [48]	MeCN	**62a**	89 (β only)
12	Fe/mont K10	MeOH	25 [48]	MeCN	**62a**	83 (β only)
13	Al/mont K10	MeOH	25 [48]	DCM	**62a**	74 (β only)
14	Fe/mont K10	MeOH	25 [48]	DCM	**62a**	68 (β only)
15	Al/mont K10	MeOH	50 [1]	MeCN	**62a**	97 (β only)
16	Fe/mont K10	MeOH	50 [1]	MeCN	**62a**	89 (β only)
17	Al/mont K10	MeOH	80 [0.5]	MeCN	**62a**	81 (β only)
18	Fe/mont K10	MeOH	80 [0.5]	MeCN	**62a**	79 (β only)
20	Al/mont K10	EtOH	50 [1]	MeCN	**63**	91 (β only)
21	Al/mont K10	*n*PrOH	50 [1]	MeCN	**64**	88 (β only)
22	Al/mont K10	*i*PrOH	50 [1]	MeCN	**66**	68 (β only)
23	Al/mont K10	*n*BuOH	50 [1]	MeCN	**65**	84 (β only)
24	Al/mont K10	BnOH	50 [1]	MeCN	**67**	87 (β only)
25	Al/mont K10	EtSH	50 [1]	MeCN	**59**	92 (α only)
26	Al/mont K10	$C_8H_{17}SH$	50 [1]	MeCN	**68**	59 (α only)

Table 3.5*: Nuclephilic substitution at C-2 performed on oxazoline derivative* ***16***

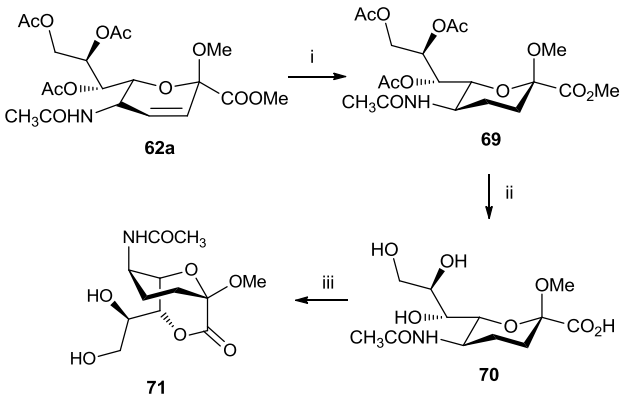
At this point we decided to perform the same reaction using BF_3Et_2O and TMSOTf as catalyst but the only product we isolated is the 4-hydroxy epimer of Neu5Ac glycal **17** (Table **3.5**, entries 7,8) with an higher amount of starting material. These experiments strongly suggest the essentiality of the

coordinating metal effect for the reaction occurrence. So, attempting to confirm our hypothesis, we carry out two reactions catalyzed by $FeCl_3$ and $AlCl_3$ respectively and the only product isolated, in both cases, was the 4-epi-chloride **57** (Table **3.5**, entries 9,10). Therefore we substituted the chlorine counterion with the Montmorillonite K-10, creating the Al/Fe-rich Montmorillonite K-10 (Table **3.5**, entries 11,12). To our delight, we obtain the desired β-methy ketoside in high yield and stereoselectivity in both cases (89% and 83%). We perform several experiments (summarized in Table **3.6**, entries 13-18) to optimize the reaction conditions, varying solvent and temperature. In all cases the reaction afforded good product yields and β selectivity, in particular we obtain the best results performing the reaction in MeCN catalyzed by Al^{3+}-Mont at 50°C (Table **3.5**, entry 15).

Successively we evaluate the extensively of this protocol using different nucleophiles (alcohols or thiols) (Table **3.5**, entries 20-26). In every reaction performed with alcohols the desired *O*-glycoside *(*Table **3.5**, entries 20-24*)* is obtained in short time, high yield, and total stereoselectivity. We afford the same good results using ethanthiol and octanthiol as nucleophiles (Table **3.5**, entries 25,26).

Since these derivatives were little considered in literature, no general method to exactly determinate the C-2 configuration is available, thus we initially perform the classical NOESY analysis on the declared β methylketosides **62a** and **62b** coupled with their preferred modeling model to identify the diagnostic NOE signals. Unfortunately, these experiments do not lead to the desired results, so we have to proceed using a new empirical method to determinate the C-2 stereochemistry. So, we set up a simple procedure involving our 1,7 lactonization reaction[102],[103] to discriminate between the two anomers, based on the evidence that just the β anomer has the carboxylated function at the right distance from the OH group at C-7 to permit the lactonization.

Thus, in sequence we reduce the 3,4 double bound of the supposed β methylketoside **62a** to obtain the compound **69**, and, after complete esteric function saponification to analogue **70**, we perform the lactonization reaction under standard condition. As expected, we obtain the desired 1,7 lactone **71**, confirming our supposition (Scheme 3.13).



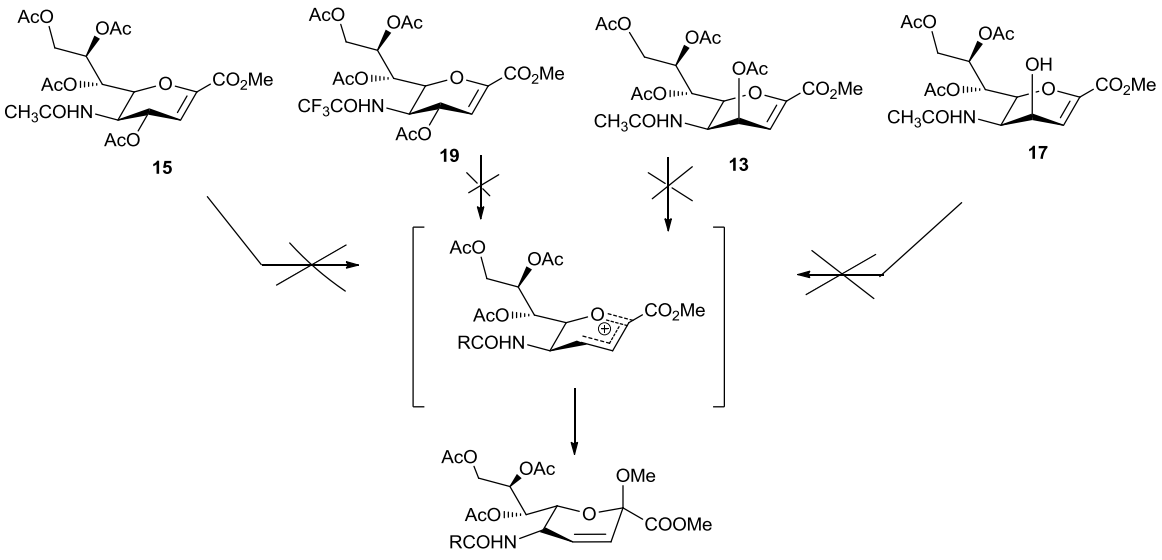
Scheme 3.13*: Lactonization reaction of the β-methylketoside* **62*a***. *Reagents and conditions:i, H_2, Pd/C in MeOH, 2 h, 25°C; ii, K_2CO_3 in MEOHaq; iii, CbzCl, TEA in DMF/THF, 0°C →25°C, 2h.*

Once attributed the 2-β conformation of compound **62a**, we can confront its ^{1}H-NMR spectra 2-α conformation of compound **62b** and find the main differences between the two spectra. Therefore, we note that in the **62a** the two hydrogens of the 3,4-double bound overlap in a unique singlet signal (δ = 5.91 ppm), while in **62b** these two hydrogens two far-between doublet of doublets signals (3-Hδ = 6.11 ; 4-Hδ = 5.72 ppm). Moreover we observe another significant variation between the chemical shift of two 6-H signals: from 4.05 ppm of the 2β configuration to the 4.37 ppm of the 2α.

Furthermore, we have to underline that we check these diagnostic variations also in the ^{1}H-NMR spectra of the two thioketosides **58** and **59**, so defining their anomeric configuration.

Actually, in order to investigate the involvement of a potential allylic carbocation, proposed by Ikeda & coworkers[76] we perform our reaction on

protected DANA **15**, FANA **19**, 4β-OAc DANA **13** and 4β-OH DANA **17** using MeOH as nucleophile. According to our expectation, in all attempts the reaction fails, in contrast to the carbocation intermediate, supporting our coordination mechanism (Scheme **3.14**).



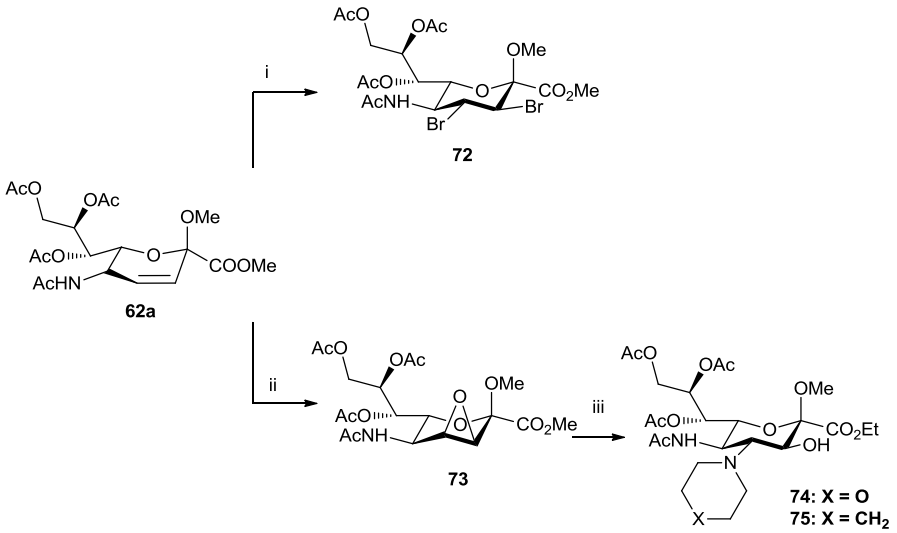
Scheme 3.14: *C-2 substitution reaction performed on glycals **15**, **19**, **13**, **17**.Reagents and conditions: MeOH, Bi(OTf)$_3$/Mont K10 in MeCN, 25°C, 48 h.*

However, at the moment, in absence of a detailed study, any attempted of rationalization appears highly speculative.

Once we set up our simple, stereocontrolled and inexpensive protocol providing the new 3,4 unsaturated *O,S*-glycosides of Neu5Ac we can totally saponificate them, to obtain new unsaturated Neu5Ac derivatives to be tested as sialidase inhibitors. However, we can also functionalize the 3,4 double bound to easily introduce new substituents both at position 3 and 4.

In fact, in some preliminary experiments, we rapidly obtain the 3,4 dibromide Neu5Ac derivative **72** and also the 3,4 epoxide analogue **73**, from the derivative **62a.**

Moreover treating compound **73** with morpholine or piperidine we straightly afford the new compounds **74** and **75** respectively (transesterificated at the ester function at C-1) (Scheme **3.15**).

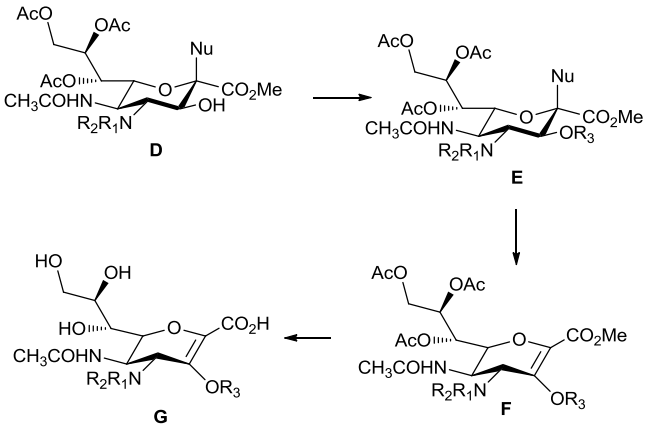


Scheme 3.15: *Derivatization of the 3,4 double bound of compound* ***62a.*** *Reagents and conditions: i, Br_2 in DMC, 25°C, 30 min; ii, mCPBA in MeCN, 80°C, 8h; iii, piperidine or morpholine in EtOH, 80°C, 12h.*

Interestingly, the nucleophilic attack on the epoxide ring occurs just to the C-4 position and from the α side of the molecule providing just and efficiently the 4α-amine, 3-hydroxy derivatives (Scheme **3.15**).

Anyway at the real moment we are accomplishing some more experiments to obtain a great number of C-3, C-4 substituted derivatives (**E,** Scheme **3.16**) to be 2,3 unsaturated (**F,** Scheme **3.16**) and hydrolyzed (**G,** Scheme **3.16**) to be finally tested as neuraminidase inhibitors.

In the end, this protocol, really shorter than the ones reported in literature by now, can represent the easiest and faster way to obtain new 3,4 substituted glycal analogues (**G,** Scheme **3.16**) as shown in scheme **3.16**.



Scheme 3.16: *Hypothetic protocol to synthesize compounds with the general formula of* ***G***

3.6 Biological assays

The newly synthesized glycals **1a-d**, **2a-d**, **11a-d** and **18b-d** are evaluated for their ability to inhibit the enzyme activity of two bacterial NAs *Vibrio cholerae* and *Clostridium perfrnigens*, of one viral NA, Newcastle disease virus, and, finally, of the mammalian Neu3 sialidase, using an *in vitro* fluorometric assay[104].The inhibitory activities of these new compounds, with comparison to the general sialidase inhibitor DANA **76a**, and previously synthesized compounds **76b-d** (figure **3.6**), DANA analogues with a perfluorinated amide at C-5, are reported in this paragraph.

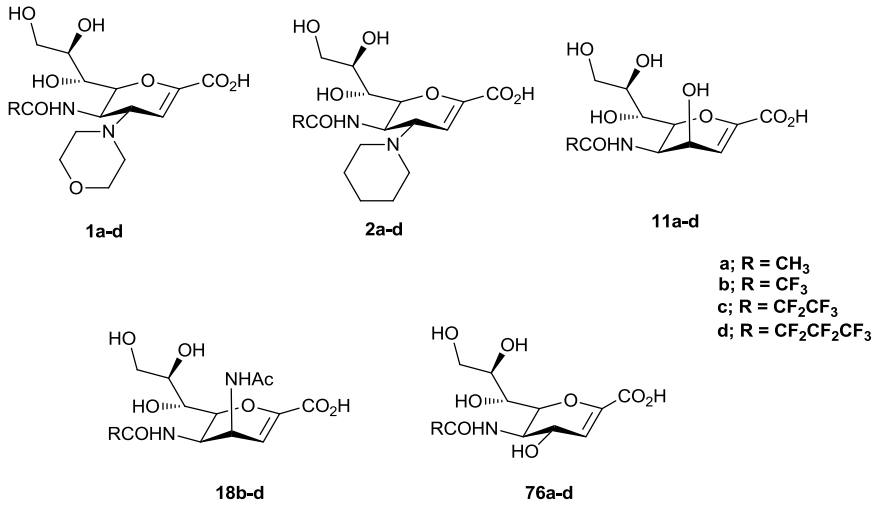


Figure 3.6: *Structures of glylcals tested in the biological assays*

3.6.1 Inhibition of *Vibrio cholerae* neuraminidase activity

As we can observe from the reported data, the most potent compound tested is **76b** (FANA), as reported in literature[60], moreover extending the perfluorinated amide at position 5 (compounds **76c** and **76d**) we can observe a reduction of the potency.

Compound	IC_{50} (10^{-5} M)[a]	Compound	IC_{50} (10^{-5} M)[a]
DANA (**76a**)	4.3	**2c**	468.0
76b	2.7	**2d**	442.0
76c	8.3	**11a**	15.8
76d	15.8	**11b**	8.0
1a	120.0	**11c**	41.9
1b	140.0	**11d**	48.2
1c	150.1	**18b**	3.7
1d	142.7	**18c**	10.4
2a	141.3	**18d**	29.4
2b	420.7		

[a]Results are given as means for three experimentally determined values; IC_{50} 95% confidence intervals are within ±15% of the IC_{50} value

A severe decrease in potency is noticed testing every compound with a cyclic amine at position C-4α (compounds **1a-d**, **2a-d**), according with the absence of the enzymatic cavity at C-4[32]. Instead, the C-4 DANA epimer **11a** shows a good value of IC_{50} (15.8 10^{-5}M), but anyway higher than DANA (IC_{50} = 4.3 10^{-5}M), and, however, we note the same trend between the *N*-perfluorinated epimers **11b-d** and the DANA derivatives **76b-d**.

We obtain a very interesting result testing the 4β*N*-acetylated compound **18b**, in agreement of the reported active site deep cavity directly below the C-4-epi position[32]. It is however less active than **76b**, and, also for this class of compounds we can observe the activity decrease, extending the amide at C-5 (compounds **76c-d**), indicating that the hydrophobic task in correspondence of

C-5 could be less large than expected[32], and cannot tolerate hindered group as the pentafluoropropionamide and heptaflurobutiramide.

3.6.2 Inhibition of *Clostridium Perfringens* neuraminidase activity

In case of *Clostridium perfringens* neuraminidase, we do not achieve any good result. In fact, after confirming the inhibition activity of DANA **76a**, the exchange of the acylamide group with a *N*-perfluorinated one leads to a complete lost of activity.

Compound	IC_{50} (10^{-5} M)[a]	Compound	IC_{50} (10^{-5} M)[a]
DANA (**76a**)	4.3	**2c**	>1000
76b	>1000	**2d**	>1000
76c	>1000	**11a**	56.8
76d	>1000	**11b**	18.1
1a	>1000	**11c**	353.6
1b	>1000	**11d**	>1000
1c	>1000	**18b**	>1000
1d	>1000	**18c**	>1000
2a	>1000	**18d**	>1000
2b	>1000		

[a]Results are given as means for three experimentally determined values; IC_{50} 95% confidence intervals are within ±15% of the IC_{50} value

In case of *Clostridium perfringens* neuraminidase, we do not achieve any good result. In fact, after confirming the inhibition activity of DANA **76a**, the exchange of the acylamide group with a *N*-perfluorinated one leads to a complete lost of activity. Similarity, we do not obtain any significant value also testing the 4-aminated and 4β-acylated derivatives **1a-d**, **2a-d**, **18b-d**. Just the C-4 epimers of DANA **11a-c** present a poor activity, that drastically decreases for the compound **11c** till to become void for the heptabutyricamide **11d**.

3.6.3 Inhibition of Newcastle disease virus (NDV) neuraminidase activity

The evaluation of the NDV neuraminidase activity gives extremely interesting results, in fact, in this case, many compounds present IC_{50} values very similar to the DANA one **76a**.

Compound	IC_{50} (10^{-5} M)[a]	Compound	IC_{50} (10^{-5} M)[a]
DANA (**76a**)	3.8	**2c**	16.3
76b	4.6	**2d**	11.4
76c	2.5	**11a**	4.7
76d	0.9	**11b**	7.1
1a	10.8	**11c**	3.1
1b	14.5	**11d**	5.1
1c	3.3	**18b**	1.8
1d	4.6	**18c**	5.2
2a	60.1	**18d**	7.4
2b	11.7		

[a]Results are given as means for three experimentally determined values; IC_{50} 95% confidence intervals are within ±15% of the IC_{50} value

In particular, if we consider the DANA series **76a-d**, we can note the decreasing of the IC_{50} values from the *N*-trifluoroacetamido derivative (**76b**) up to the most active compound **76d** with a *N*-heptafluorobutyramide at C-5 position (IC_{50} = 0.9 10^{-5} M). Probably this enhancement in the inhibitory activity indicates that larger amide can make stronger hydrophobic interactions with the enzyme active site task in correspondence of C-5 position of sialic acid. Also the compounds with the cyclic amines work well, in detail the morpholine set **1a-d** seems to inhibit better than the piperidene one **2a-d**. As far as the DANA epimers **11a-d** concerned, they have an activity just slightly inferior respect the DANA series (**76a-d**), with interesting results, especially the pentapropionamide derivative **11c (**IC_{50} = 3.1 10^{-5} M). To

conclude, in the 4*N*-acetylated series **18b-d** we can find the very good data of the trifluoroacetamido glycal **18b (**IC_{50} = 1.8 10^{-5} M), this inhibitor activity gradually decreases extending the C-5 amide.

3.6.4 Inhibition of murine Neu3 sialidase activity

This biological assay, conducted on the cell membrane proteins, presents IC_{50} values that increase in DANA series **76a-d**, from the 2.29 10^{-5} M of DANA **76a** to the complete inactivity of the highest derivative **11d**.

Compound	IC_{50} (10^{-5} M)[a]	Compound	IC_{50} (10^{-5} M)[a]
DANA (**76a**)	2.3	**2c**	>1000
76b	10.1	**2d**	>1000
76c	37.3	**11a**	>1000
76d	482.1	**11b**	>1000
1a	>1000	**11c**	>1000
1b	>1000	**11d**	>1000
1c	>1000	**18b**	4.3
1d	>1000	**18c**	>1000
2a	>1000	**18d**	>1000
2b	>1000		

[a]Results are given as means for three experimentally determined values; IC_{50} 95% confidence intervals are within ±15% of the IC_{50} value

Regarding the other compounds, no significant activity is detected, apart from the 4β-acylamino analogue **18b**, that shows a very good value of IC_{50} of 4.3 10^{-5} M.

In summary, we can conclude that bacterial neuraminidases of *Vibrio cholerae* and *Clostridium prefringens* probably do not present the same hydrophobic cavity, with the same characteristics of viral neuraminidases in correspondence of C-4α position[105], as we can observe from the drop of activity in both cases, testing the C-4 aminated compounds **1a-d**, **2a-d**. Moreover, whereas the FANA derivative **76b** is the most active compound in case of *Vibrio cholerae* NA, it is completely inactive against *Clostridium prefringens* NA, as the higher analogues **76b-d**. The only scarcely active compounds against *Clostridium prefringens* sNA are the C-4 DANA epimers (**11a-c**), where the most potent is the **11b** with an IC_{50} value of 18 10^{-5} M. The 4β-*N*-acetylated compounds active against *Vibrio cholerae* sialidase, do not show any inhibition against *Clostridium prefringens* NA. In particular, the 5-*N*-trifluoracetamide derivative **18b** is a quite strong inhibitor of *Vibrio cholerae* NA (IC_{50} = 3.7 10^{-5} M), probably underlying the presence of hydrophobic interaction of its C-4β acetamido group with a cavity in the enzyme active site, not exhibited in *Clostridium prefringens* NA. This encouraging result in case of *Vibrio cholerae* NA shows that the strategy of filling this cavity with a hydrophobic group in correspondence of C-4β position of sialic acid should be explored further. A similar observation can be done for mammalian Neu3 sialidase, where the inhibition activity decreases in DANA series **76a-d** extending the amide at C-5 (form **76a**: IC_{50} = 2.3 10^{-5} M, to **76d**: IC_{50} = 482.1 10^{-5} M). Also in this case, no compound is found to be active, except for the C-4β acetamido glycal **18b**.

Saving the best for the last, we now consider NDV inhibition data, starting from the DANA series **76a-d**. We can observe an activity inverse tendency respect C-5 position, in fact the heptafluorobutiricamide derivative **76d** displays more than four-fold greater inhibitor activity than Neu5Ac2e **76a**, and it is the most potent compound tested in our experiments. This C-5 activity tendency is not strictly followed in the other series, but, however, it results clear that a quite hindered substituent is tolerated at position C-5.

Nevertheless, we have to notice that in every experiments we conduct, exists an equilibrium between the C-4 and C-5 substituents, and we cannot examine these two positions separately. Indeed, also in case of NVD NA, a very interesting IC_{50} value ($1.8\ 10^{-5}$M) is observed for the C-4β acetamido glycal **18b**, that increase extending the C-5 amide. Finally, these are the unique assays where C-4α-aminated series **1a-d**, **2a-d** display interesting IC_{50} values; this fact is probably due to the interactions between the C-4α substituent and an enzymatic hydrophobic pocket in correspondence of this position, according to the literature[106].

4 CONCLUSION AND FUTURE PERSPECT

4. Conclusion and future perspects

This Ph.D. work represents part of a larger research project directed to study the correlation between the different structures of unsaturated Neu5Ac derivatives and their inhibitory activity against viral, bacterial and mammalian sialidases. In fact we are interested in finding the general features to define a potent and selective inhibitor of a particular kind of sialidase, to be used as pharmaceutical or biochemical tools. So, after a careful literature investigation, we set up new synthetic protocols to rapidly and systematically modify a specific molecular portion of DANA structure, and we evaluate the biological activity linked to this modification.

Focusing on this particular Ph.D. work, we have set up many rapid, simple and efficient protocols to introduce new substituents into the DANA structure, starting from the synthesis of new C-4α aminated derivatives **1a-d** and **2a-d**. Then we synthesize for the first time the *N*-perfluorinated C-4 epimers of DANA (**11b-d**), clarifying also their mechanism formation and the key role of the oxazoline derivative **16**. This study is propaedeutical to the successive Ritter like reaction performed for the first time on Neu5Ac derivatives. Thanks to this reaction we obtain the new 4-*N*β-acetylamide analogues **18a-d** straightly and in a very good yields. Successively, we extend our research formulating a general nucleophilic substitution at C-4 position of the Neu5Ac glycal, synthesizing a great number of compounds by the rapid introduction of alcohols, thiols, sulfonamides and alogens. Moreover, we deeply analyze this reaction, founding a probable rationalization to explain its stereo- and the region-selectivity. In the end we open the doors to the synthesis of new 3,4 unsaturated alkylglycosides of Neu5Ac, utilizing a nucleophilic substitution directed, this time, to the C-2 position of neuraminic acid catalyzed by a practical end economical Al^{3+}-enriched resin. These new compounds are particularly interesting not just because present a novel Neu5Ac skeleton and they have not been biologically tested so far, but also because they represent

the suitable intermediates to one-pot derivatize C-3 and C-4 positions of sialic acid. In fact, as shown in our preliminary results, we can easily form an epoxide derivative **73** that can suffer a new nucleophilic attack. As example of products of this synthetic pathway, we obtain the 3- hydroxyl, 4- morpholine or piperidine derivatives **74** and **75**, in high yields and total stereo- and regio-selectivity. Obviously, we propose to afford an high number of compounds C-3 and C-4 substituted to be successively unsaturated and saponificated to be finally tested against influenza A virus neuraminidase and other aforementioned sialidase types.

Then, we perform the preliminary biological assays of part of the obtained compounds, more precisely of glycals **1a-d**, **2a-d**, **11a-d** and **18b-d**, against *Vibrio cholerae*, NDV, *Clostridium perfringens* and mammalian Neu3 sialidases. We obtain interesting results especially regarding the NDV neuraminidase inhibition data, that suggest the possibility to design a NDV NA selective inhibitor by tuning the substituent at C-5 position.

In the future, we propose to saponificate the obtained compounds described paragraph **3.3** and **3.4** and to test their inhibition activity against the aforementioned sialidases. Once collected these data and integrated to the actual results of this work, we will plan how to develop our research.

5. EXPERIMENTAL

5. Experimental

5.1 Materials

All chemicals used were special (specific) grade unless otherwise specified.

Water was prepared by filtering deionized water on a Milli-Q Simplicity 185 filtration system from Millipore (Bedford, MA, USA).

Discovery DSC-18 SPE tubes for sample clean-up were purchased from Supelco (Bellefonte, PA, USA).

Solvents were dried using standard methods and distilled before use. The reactions are thermostated by block heater -BBA- Grant Boekel apparatus. The progress of all reactions was monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F_{254}) using UV light, 50% sulphuric acid, anisaldehyde/H_2SO_4/EtOH solution or 0.2% ninhydrin in ethanol and heat as developing agent. All flash chromatography was performed with normal phase silica gel (E. Merck 230-400 mesh silica gel), following the general protocol of Still[1]. GLC was performed by Hewlett 5890 PACKARD Series II using HP-5 30 m x 0.32 mm, 0.25 µm film-thickness column. Melting points were measured on a SMP3 mp apparatus (Stuart Scientific, USA) and are not corrected. NMR spectra were recorded at 25°C on a Bruker AM-500 spectrometer operating at 500.13 MHz for ^1H and 125.76 MHz for ^{13}C. The chemical shifts are reported in ppm and coupling constant are given in Hz, relative to CD_3OD signal fixed at 3.31 ppm for ^1H spectra and to CD_3OD signal fixed at 49.05 ppm for ^{13}C spectra, relative to $CDCl_3$ signal fixed at 7.26 ppm for ^1H spectra and to $CDCl_3$ signal fixed at 77.00, relative to CD_3CN signal fixed at 1.94 ppm for ^1H spectra and to CD_3CN signal fixed at 1.24 ppm for ^{13}C spectra. Proton and carbon assignments were established, if necessary, with ^1H-^1H and ^1H-^{13}C correlated NMR experiments. Data for ^1H NMR are recovered as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br , broad), coupling constant(s) in Hz, number of protons, assignment of proton(s). In some cases, reported in the

text, the ^{1}H NMR inspection was performed on the total reaction mixture using CD_3CN as a solvent. Optical rotations were taken on a Perkin-Elmer 241 polarimeter equipped with a 1 dm tube; $[a]_D$ values are given in 10^{-1}deg cm^2 g^{-1} and the concentration are given in g/100 mL.

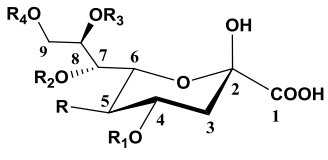
Mass spectrometry was performed using Finnigan LCQ_{Deca} quadrupole ion-trap mass spectrometer equipped with an ESI ion source (Finnigan ThermoQuest, San Jose, CA, USA). The spectra were collected in continuous flow mode by connecting the infusion pump directly to the ESI source. Solutions of compounds were infused at a flow rate of 5 mL/min. The spray voltage was set at 5.0 kV in the positive and at 4.5 kV in the negative ion mode with a capillary temperature of 220 °C. Full-scan mass spectra were recorded by scanning a m/z range of 100-2000.

Work-up refers to successive washing of the organic layer with an ice cold aqueous $NaHCO_3$ saturated solution and water, to drying over Na_2SO_4, and evaporation of the solvent under reduced pressure.

5.2 General method

Nuclear magnetic resonance (NMR) spectra were recorded at 25°C on a Bruker AM-500 spectrometer operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. The chemical shifts are reported in ppm and coupling constant are given in Hz, relative to CD_3OD signal fixed at 3.31 ppm for ^{1}H spectra and to CD_3OD signal fixed at 49.05 ppm for ^{13}C spectra, relative to $CDCl_3$ signal fixed at 7.26 ppm for ^{1}H spectra and to $CDCl_3$ signal fixed at 77.00, relative to CD_3CN signal fixed at 1.94 ppm for ^{1}H spectra and to CD_3CN signal fixed at 1.24 ppm for ^{13}C spectra and to internal $(CH_3)_3COH$ 1.24 ppm and 30.29 ppm for solutions in D_2O. Proton and carbon assignments were established, if necessary, with ^{1}H-^{1}H and ^{1}H-^{13}C correlated NMR experiments. Data for ^{1}H NMR are recovered as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br , broad), coupling constant(s) in Hz, number of protons, assignment of proton(s). In some cases, reported in the

text, the ^1H NMR inspection was performed on the total reaction mixture using CD_3CN as a solvent. The carbon numeration used in the NMR description is that given in figure.



All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F_{254}) using UV light, 50% sulfuric acid, anisaldehyde/H_2SO_4/EtOH solution and heat as developing agent. E. Merck 230–400 mesh silica gel was used for flash column chromatography.

Optical rotations were taken on a Perkin-Elmer 241 polarimeter equipped with a 1 dm tube; $[a]_D$ values are given in 10^{-1}deg cm^2 g^{-1} and the concentration are given in g/100 mL.

I

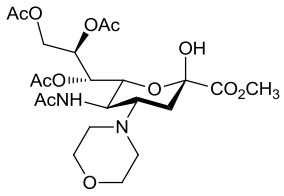
Mass spectra were carried out using a Finnigan LCQdeca (ThermoQuest) ion trap mass spectrometer equipped with an electrospray source (ESI). The spectra were collected in continuous flow mode by connecting the infusion pump directly to the ESI source. Solutions of compounds were infused at a flow rate of 10 µL/min. The spray voltage was set at 5.0 kV, operating in the positive ionization mode with capillary temperature of 220°C. Full-scan mass spectra were recorded by scanning a m/z range of 150–2000.

The GC separation was performed on a Finningan Trace GC ultra gas chromatograph equipped with a 15 m-0.25 mm RTX-5MS capillary column, 0.25 mm film phase (RESTEC, USA). The column was coupled to a Finnigan Trace DSQ spectrometer (mass detection limit 1050). The analyses were performed routinely in the electron impact mode (ionisation energy 70 eV, source temperature 150 °C).

5.3 Reference standards synthesized

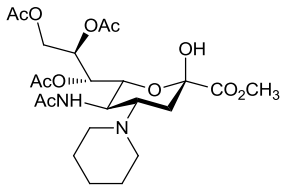
5.3.1. Synthesis of C-4α aminated derivatives of normal and *N*-perfluorinated Neu5Ac2en

Preparation of methyl 5-acetamido-4-(morpholin-4-yl)-7,8,9-tri-O-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (4a)



The ester **3** (213 mg, 0.40 mmol) and morpholine (0.35 mL, 4.0 mmol) are reacted in pyridine, according to the procedure reported by Liu et al.[79], to afford the title compound **4a**, as a white solid (168 mg, 81%): m.p. 153–155 °C; $[\alpha]_D$ + 26.6 (c 1 in $CHCl_3$); (Found: C, 50.88; H, 6.58; N, 5.60; Calc. for: $C_{22}H_{34}N_2O_{12}$ C, 50.96; H, 6.61; N, 5.40%); ^{1}H NMR ($CDCl_3$) δ 5.35 (1H, dd, $J_{7,8}$ = 6.1, $J_{7,6}$ = 2.1 Hz, 7-H), 5.34–5.26 (1H, br s, N–H), 5.23–5.17 (1H, m, 8-H), 4.66 (1H, dd, $J_{9a,9b}$ = 12.3, J9a,8 = 2.0 Hz, 9a-H), 5.29 (1H, br s, OH), 4.13–4.06 (2H, overlapping, 5-H and 6-H), 3.99 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.5 Hz, 9b-H), 3.85 (3H, s, $COOCH_3$), 3.66–3.52 (4H, overlapping, $N(CH_2CH_2)_2O$), 2.92 (1H, br s, 4-H), 2.68–2.63 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.44–2.25 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.11 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.04–1.98 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.94 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.5, 170.4, 170.2 (4C, 3XCH_3COO and $NHCOCH_3$), 169.8 (C-1), 95.1 (C-2), 72.3 (C-6), 71.3 (C-8), 68.6 (C-7), 67.5 (2C, N $(CH_2CH_2)_2O$), 62.8 (C-9), 61.6 (C-4), 53.4 ($COOCH_3$), 48.6 (2C, $N(CH_2CH_2)_2O$), 46.3 (C-5), 29.5 (C-3), 23.2 ($NHCOCH_3$), 20.9, 20.8, 20.7 (3C, 3XCH_3COO); MS (ESI positive) m/z 518.9 $[M + H]^+$, 541.1, $[M + Na]^+$, 1058.8 $[2M + Na]^+$. An α-aminated compound **4a**, with correct elemental analyses and physico-chemical properties superimposable to that above described is also obtained in 80% yields when the reaction is performed in CH_3CN (4 mL), heating at 60 °C for 5 h.

Preparation of methyl 5-acetamido-4-(piperidin-1-yl)-7,8,9-tri-O-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (5a)



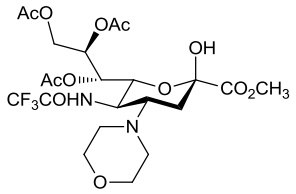
The ester **3** (362 mg, 0.68 mmol) and piperidine (0.68 mL, 6.8 mmol) are reacted in pyridine, according to the procedure reported by Liu et al.[79], to afford the title compound **5a**, as a white solid (270 mg, 76%): m.p. 132–133 °C; $[\alpha]_D$ + 20.3 (c 1 in $CHCl_3$); (Found: C, 53.14; H, 6.93; N, 5.32; Calc. for: $C_{23}H_{36}N2O_{11}$ C, 53.48; H, 7.02; N, 5.42%); ^{1}H NMR ($CDCl_3$) δ 5.38 (1H, d, $J_{NH,5}$ = 9.9 Hz, N–H), 5.32 (1H, br d, $J_{7,8}$ = 6.3 Hz, 7-H), 5.24–5.20 (1H, m, 8-H), 4.43 (1H, br d, $J_{9a,9b}$ = 12.2 Hz, 9a-H), 4.14–4.08 (2H, overlapping, OH and 5-H), 4.03 (1H, br d, $J_{6,5}$ = 10.0 Hz, 6-H), 4.00 (1H, dd, $J_{9b,9a}$ = 12.2, $J_{9b,8}$ = 7.3 Hz, 9b-H), 3.85 (3H, s, COOCH3), 3.02–2.97 (1H, m, 4-H), 2.67–2.59 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.34–2.26 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.10 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 2.02–1.95 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.95 (3H, s, CH_3CONH), 1.54–1.31 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR (CDCl3) δ 170.7, 170.6, 170.5, 170.4 (4C, 3XCH_3COO and NHCOCH3), 170.0 (C-1), 95.3 (C-2), 72.7 (C-6), 71.4 (C-8), 68.7 (C-7), 62.9 (C-9), 61.8 (C-4), 53.2 ($COOCH_3$), 49.5 (2C, N $(CH_2CH_2)_2CH_2$), 46.8 (C-5), 29.5 (C-3), 26.5 (2C, N $(CH_2CH_2)_2CH_2$), 24.6 (1C, $N(CH_2CH_2)_2CH_2$), 23.2 ($NHCOCH_3$), 21.0, 20.9, 20.8 (3C, 3XCH_3COO); MS (ESI positive) m/z 517.1 $[M + H]^+$, 539.1, $[M + Na]^+$, 1054.8 $[2M + Na]^+$. α-aminated compound **5a**, with correct elemental analyses and physico-chemical properties superimposable to that above described is also obtained in 73% yields when the reaction is performed in CH_3CN (4 mL) heating at 60 °C for 7 h.

Amination at the C-4 α-position of the *N*-perfluoroacylatedneuraminic acid methyl esters 8b-d.

General procedure.

The appropriate *N*-perfluoracylated derivative20 (0.40 mmol), dissolved in CH_3CN (4 mL) is reacted with the appropriate cyclic amine (4.0 mmol) under stirring at 60 °C, for the reported time. Then, the solvent is evaporated under reduced pressure, and the residue is purified by rapid chromatography.

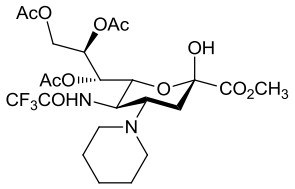
Preparation of methyl 5-(2,2,2-trifluoroacetamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (4b)



Starting with the amido ester **8b**[80] (235 mg, 0.40 mmol) and morpholine (0.35 mL, 4.0 mmol) the amino derivative **4b** is obtained, after 5 h, as a white solid (181 mg, 79%): m.p. 149–151 °C; $[\alpha]_D$ + 23.6 (c 1 in $CHCl_3$); (Found: C, 46.26; H, 5.48; N, 4.96; Calc. for: $C_{22}H_{31}F_3N2O12$ C, 46.16; H, 5.46; N, 4.89%); ^{1}H NMR (CDCl3) δ 6.78 (1H, d, $J_{NH,5}$ = 10.2 Hz, N–H), 5.34 (1H, dd, $J_{7,8}$ = 4.7, $J_{7,6}$ = 2.4 Hz, 7-H), 5.28 (1H, ddd, $J_{8,9b}$ = 7.3, $J_{8,7}$ = 4.7, $J_{8,9a}$ = 2.4 Hz, 8-H), 4.74 (1H, br s, OH), 4.55 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.4 Hz, 9a-H), 4.30 (1H, dd, $J_{6,5}$ = 10.3, J6,7 = 2.4 Hz, 6-H), 4.06–3.98 (2H, overlapping, 5-H and 9b-H), 3.87 (3H, s, $COOCH_3$), 3.65–3.50 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.00–2.96 (1H, m, 4-H), 2.71–2.65 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.33–2.27 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.17 (3H, s, CH_3COO), 2.09–1.98 (8H, overlapping, 2XCH_3COO, 3a-H and 3b-H); ^{13}C NMR ($CDCl^3$) δ 171.2, 170.8, 169.8 (3C, 3XCH_3COO), 169.4 (C-1), 157.8

(q, $J_{C,F}$ = 37 Hz, $COCF_3$), 122.0–110.0 (1C, CF_3), 95.1 (C-2), 71.4 (C-6 and C-8), 68.7 (C-7), 67.1 (2C, N (CH2CH2)2O), 62.6 (C-9), 60.9 (C-4), 53.4 ($COOCH_3$), 48.7 (2C, $N(CH_2CH_2)_2O$), 46.8 (C-5), 29.4 (C-3), 20.8, 20.7, 20.5 (3C, $3XCH_3COO$); MS (ESI positive) m/z 573.2 $[M + H]^+$, 595.1 $[M + Na]^+$, 1166 $[2M + Na]^+$.

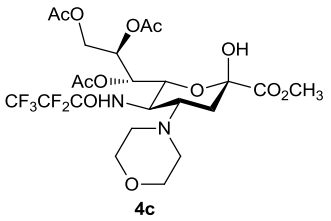
Preparation of methyl 5-(2,2,2-trifluoroacetamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (5b)



5b

Starting with the amido ester **8b**[80] (235 mg, 0.40 mmol) and piperidine (0.40 mL, 4.0 mmol) the amino derivative **5b** is obtained, after 7 h, as a white solid (171 mg, 75%): m.p. 160–162 °C; $[\alpha]_D$ + 36.3 (c 1 in CHCl3); (Found: C, 48.37; H, 5.89; N, 4.79; Calc. for: $C_{23}H_{33}F_3N_2O_{11}$ C, 48.42; H, 5.83; N, 4.91%); ^{1}H NMR ($CDCl_3$) δ 6.65 (1H, br s, N–H), 5.33 (1H, dd, $J_{7,8}$ = 4.6, $J_{7,6}$ = 2.1 Hz, 7-H), 5.32–5.28 (1H, m, 8-H), 4.66 (1H, br s, OH), 4.55 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9,8}$ = 2.2 Hz, 9a-H), 4.27 (1H, dd, $J_{6,5}$ = 10.2, $J_{6,7}$ = 2.1 Hz, 6-H), 4.09–3.98 (2H, overlapping, 5-H and 9b-H), 3.86 (3H, s, $COOCH_3$), 2.96–2.92 (1H, m, 4-H), 2.63–2.57 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.26–2.19 (2H, overlapping, $N(CH_2CH_2)_2$-CH_2), 2.14 (3H, s, CH_3COO), 2.09 (3H, s, CH_3COO), 2.06–1.95 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.55–1.32 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); $_{13}$C NMR ($CDCl_3$) δ 171.3, 170.9, 170.0 (3C, $3XCH_3COO$), 169.8 (C-1), 157.8 (q, $J_{C,F}$ = 37 Hz, $COCF_3$), 120.0–110.0 (1C, CF_3), 95.4 (C-2), 72.0 (C-6), 71.7 (C-8), 69.0 (C-7), 62.7 (C-9), 61.4 (C-4), 53.4 ($COOCH_3$), 49.8 (2C, $N(CH_2CH_2)_2CH_2$), 47.3 (C-5), 29.6 (C-3), 26.5 (2C, $N(CH_2CH_2)_2CH_2$), 24.7 (1C, N $(CH_2CH_2)_2CH_2$), 20.9, 20.8, 20.7 (3C, $3XCH_3COO$); MS (ESI positive) m/z 571.2 $[M + H]^+$, 593.1 $[M + Na]^+$, 1162.6 $[2M + Na]^+$.

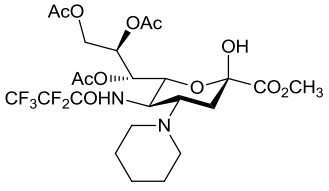
Preparation of methyl 5-(2,2,3,3,3-pentafluoropropionamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (4c).



4c

Starting with the amido ester **8c**[80] (255 mg, 0.40 mmol) and morpholine (0.35 mL, 4.0 mmol) the amino derivative **4c** is obtained, after 5 h, as a white solid (204 mg, 82%): m.p. 154–156 °C; $[\alpha]_D$ + 23.6 (c 1 in $CHCl_3$); (Found: C, 44.58; H, 4.82; N, 4.38; Calc. for: $C_{23}H_{31}F_5N_2O_{12}$ C, 44.38; H, 5.02; N, 4.50%); ^{1}H NMR ($CDCl_3$) δ 6.63 (1H, d, $J_{NH,5}$ = 10.0 Hz, N–H), 5.33 (1H, dd, $J_{7,8}$ = 5.5, $J_{7,6}$ = 2.2 Hz, 7-H), 5.25 (1H, ddd, $J_{8,9b}$ = 7.6, $J_{8,7}$ = 5.5, $J_{8,9a}$ = 2.3 Hz, 8-H), 4.67 (1H, br s, OH), 4.50 (1H, dd, $J_{9a,9b}$ = 12.3, J9a,8 = 2.3 Hz, 9a-H), 4.27 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.2 Hz, 6-H), 4.06–3.97 (2H, overlapping, 5-H and 9b-H), 3.87 (3H, s, $COOCH_3$), 3.64–3.52 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.01 (1H, ddd, $J_{4,3a}$ = $J_{4,5}$ = 11.2, $J_{4,3b}$ = 4.7 Hz, 4-H), 2.71–2.64 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.36–2.30 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.12 (3H, s, CH_3COO), 2.09–1.99 (8H, overlapping, 2XCH_3COO, 3a-H and 3b-H); ^{13}C NMR ($CDCl_3$) δ 171.4, 171.2, 170.0 (3C, 3XCH_3COO), 169.3 (C-1), 158.3 (t, $J_{C,F}$ = 26 Hz; $COCF_2CF_3$), 120.0–108.0 (2C, CF_2CF_3), 95.4 (C-2), 71.6 (2C, C-6 and C-8), 69.1 (C-7), 67.2 (2C, $N(CH_2CH_2)_2O$), 62.8 (C-9), 60.9 (C-4), 53.7 ($COOCH_3$), 49.0 (2C, $N(CH_2CH_2)_2O$), 47.4 (C-5), 29.4 (C-3), 20.9, 20.8, 20.7 (3C, 3XCH_3COO); MS (ESI positive) m/z 623.6 $[M + H]^+$, 646.7 $[M + Na]^+$, 1268.1 $[2M + Na]^+$.

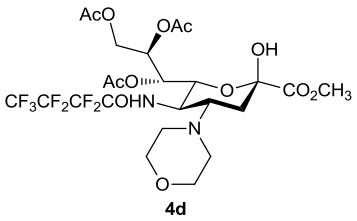
Prparation of methyl 5-(2,2,3,3,3-pentafluoropropionamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (5c).



5c

Starting with the amido ester **8c**[80] (255 mg, 0.40 mmol) and piperidine (0.40 mL, 4.0 mmol) the amino derivative **5c** is obtained, after 7 h, as a white solid (176 mg, 71%): m.p. 151–153 °C; $[\alpha]_D$ + 23.0 (c 1 in $CHCl_3$); (Found: C, 46.51; H, 5.26; N, 4.42; Calc. for: $C_{24}H_{33}F_5N_2O_{11}$ C, 46.45; H, 5.36; N, 4.51%); ^{1}H NMR ($CDCl_3$) δ 6.92 (1H, d, $J_{NH,5}$ = 8.2 Hz, N–H), 5.31 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 2.1 Hz, 7- H), 5.29–5.26 (1H, m, 8-H), 4.76 (1H, br s, OH), 4.58 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.2 Hz, 9a-H), 4.30 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.1 Hz, 6-H), 4.14–4.05 (1H, m, 5-H), 4.00 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.7 Hz, 9b-H), 3.85 (3H, s, $COOCH_3$), 2.94 (1H, ddd, $J_{4,3a}$ = $J_{4,5}$ = 11.0 Hz, $J_{4,3b}$ = 5.0 Hz, 4-H), 2.63–2.53 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.23–2.16 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.13 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.03–1.86 (5H, overlapping, CH_3COO, 3a-H and 3b- H), 1.52–1.31 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 171.7, 170.9, 169.9 (3C, 3XCH_3COO), 169.8 (C-1), 158.3 (t, $J_{C,F}$ = 27 Hz; $COCF_2CF_3$), 120.0–110.0 (2C, CF_2CF_3), 95.4 (C-2), 72.3 (C-6), 72.0 (C-8), 69.2 (C-7), 62.7 (C-9), 61.1 (C-4), 53.3 ($COOCH_3$), 49.8 (2C, $N(CH_2CH_2)_2CH_2$), 47.0 (C-5), 29.3 (C-3), 26.2 (2C, $N(CH_2CH_2)_2CH_2$), 24.7 (1C, $N(CH_2CH_2)_2CH_2$), 20.8, 20.7, 20.6 (3C, 3XCH3COO); MS (ESI positive) m/z 621.2 $[M + H]^+$, 643.7 $[M + Na]^+$, 1264.1 $[2M + Na]^+$.

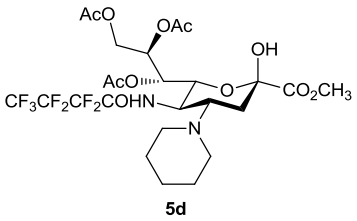
Preparation of methyl 5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-β-D-*glycero*-D-*galacto*-non-2-ulopyranosidonate (4d).



4d

Starting with the amido ester **8d**[80] (275 mg, 0.40 mmol) and morpholine (0.35 mL, 4.0 mmol) the amino derivative **4d** is obtained, after 5 h, as a white solid (215 mg, 80%): m.p. 148–150 °C; $[\alpha]_D$ + 36.5 (c 1 in $CHCl_3$); (Found: C, 42.38; H, 4.76; N, 4.28; Calc. for: $C_{24}H_{31}F_7N_2O_{12}$ C, 42.86; H, 4.65; N, 4.17%); ^{1}H NMR ($CDCl_3$) δ 6.87 (1H, d, $J_{NH,5}$ = 9.8 Hz, N–H), 5.31 (1H, dd, $J_{7,8}$ = 4.8, $J_{7,6}$ = 2.3 Hz, 7- H), 5.26 (1H, ddd, $J_{8,9b}$ = 7.3, $J_{8,7}$ = 4.8, $J_{8,9a}$ = 2.3 Hz, 8-H), 4.80 (1H, br s, OH), 4.57 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.3 Hz, 9a-H), 4.31 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.3 Hz, 6-H), 4.06–4.02 (1H, m, 5-H), 3.99 (1H, dd, $J_{9b,9a}$ = 12.4, J9b,8 = 7.3 Hz, 9b-H), 3.87 (3H, s, $COOCH_3$), 3.63–3.51 (4H, overlapping, N $(CH_2CH_2)_2O$), 3.00 (1H, ddd, $J_{4,3a}$ = J_{4-5} = 11.0, $J_{4,3b}$ = 5.3 Hz, 4-H), 2.71–2.65 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.32–2.27 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.13 (3H, s, CH_3COO), 2.09–1.99 (8H, overlapping, 2XCH_3COO, 3a-H and 3b-H); ^{13}C NMR ($CDCl_3$) δ 171.2, 171.0, 169.8 (3C, 3XCH_3COO), 169.7 (C-1), 158.2 (t, $J_{C,F}$ = 26 Hz; $COCF_2CF_2CF_3$), 121.0–108.0 (3C, $CF_2CF_2CF_3$), 95.3 (C-2), 71.4 (2C, C-6 and C-8), 69.0 (C-7), 67.0 (2C, $N(CH_2CH_2)_2O$), 62.6 (C-9), 60.7 (C-4), 53.5 ($COOCH_3$), 48.8 (2C, $N(CH_2CH_2)_2O$), 47.2 (C-5), 29.2 (C-3), 20.7, 20.6, 20.5 (3C, 3XCH_3COO); MS (ESI positive) m/z 673.1 $[M + H]^+$, 695.0 $[M + Na]^+$.

Preparation of methyl 5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-β-D-*glycero*-D-*galacto*-non-2-ulopyranosidonate. (5d)



5d

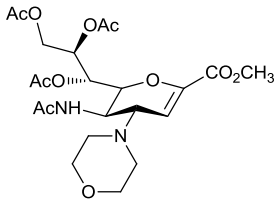
Starting from the amido ester **8d**[80] (255 mg, 0.40 mmol) and piperidine (0.40 mL, 4.0 mmol) the amino ester **5d** is obtained, after 7 h, as a white solid (190 mg, 71%): m.p. 140–142 °C; $[\alpha]_D$ + 31.2 (c 1 in $CHCl_3$); (Found: C, 44.34; H, 4.81; N, 4.24; Calc. for: $C_{25}H_{33}F_7N_2O_{11}$ C, 44.78; H, 4.96; N 4.18%); ^{1}H NMR ($CDCl_3$) δ 7.12 (1H, br s, N–H), 5.32–5.26 (2H, overlapping, 7-H and 8-H), 4.95 (1H, br s, OH), 4.63 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9,8}$ = 2.4 Hz, 9a-H), 4.32 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.2 Hz, 6-H), 4.18–4.04 (1H, m, 5-H), 3.99 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 7.4 Hz, 9b-H), 3.84 (3H, s, $COOCH_3$), 2.97–2.90 (1H, m, 4-H), 2.62–2.54 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.22–2.15 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.13 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.03–1.96 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.50–1.30 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 171.7, 170.0, 169.9 (3C, 3XCH_3COO), 169.8 (C-1), 158.3 (t, $J_{C,F}$ = 27 Hz; $COCF_2CF_2CF_3$), 120.0–105.0 (3C, $CF_2CF_2CF_3$), 95.4 (C-2), 72.3 (C-6), 72.0 (C-8), 69.3 (C-7), 62.6 (C-9), 60.9 (C-4), 53.3 ($COOCH_3$), 49.7 (2C, $N(CH_2CH_2)_2CH_2$), 47.2 (C-5), 29.3 (C-3), 26.2 (2C, $N(CH_2CH_2)_2CH_2$), 24.7 (1C, $N(CH_2CH_2)_2CH_2$), 20.7 (CH_3COO), 20.6 (2C, 2XCH_3COO); MS (ESI positive) m/z 671.2 $[M + H]^+$, 693.1 $[M + Na]^+$, 1362.6 $[2M + Na]^+$.

Dehydration of 4α-aminated 2-hydroxy peracetylated sialic acid esters 6a-d and 7a-d.

General procedure

The 2-hydroxy peracetylated sialic acid amino ester (0.20 mmol), dissolved in CH_3CN (0.60 mL), was reacted with the TFAA (84 µL, 0.6 mmol) at 23 °C for 10 min. Then, the solvent and the excess TFAA were evaporated under a nitrogen flow and the crude was dissolved in CH_3CN (0.60 mL) and treated with TFA (46 µL, 0.6 mmol) at 135 °C for 5 min, in a sealed tube. The reaction mixture was cooled, diluted with methanol (0.20 mL) and evaporated under reduced pressure. The obtained crude residue was purified by rapid chromatography, to afford the appropriate glycal.

Preparation of methyl 5-acetamido-2,6-anhydro-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (6a)

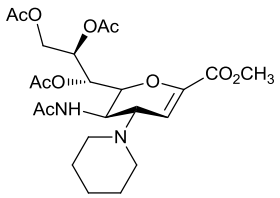


6a

The 2-hydroxy ester **4a** (104 mg, 0.20 mmol) dissolved in CH_3CN (0.60 mL), is reacted with the TFAA (84 µL, 0.6 mmol) at 23 °C for 10 min. Then, the solvent and the excess TFAA are evaporated under a nitrogen flow to afford a crude residue that is recovered in CH_3CN (0.60 mL) and treated with TFA (46 µL, 0.6 mmol) at 135 °C for 5 min, in a sealed tube. Then, the reaction mixture is cooled, diluted with methanol (0.20 mL) and evaporated under reduced pressure. The obtained crude product (88 mg) is then purified by rapid chromatography, to afford the appropriate glycal **6a**, as a white solid, (80 mg, 80%): m.p. 110–112 °C; $[\alpha]_D$ + 65.0 (c 1 in $CHCl_3$); (Found: C, 52.76; H, 6.15; N, 5.79; Calc. for: $C_{22}H_{32}N_2O_{11}$ C, 52.79; H, 6.44; N, 5.60%); ^1H NMR ($CDCl_3$) δ 6.10 (1H, d, $J_{3,4}$ = 3.1 Hz, 3-H), 5.57 (1H, dd, $J_{7,8}$ = J7,6 = 4.0 Hz, 7-H), 5.55–5.38 (1H,

br s, N–H), 5.35 (1H, ddd, $J_{8,9b}$ = 7.2, $J_{8,7}$ = 4.0, $J_{8,9a}$ = 3.3 Hz, 8-H), 4.62 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.3 Hz, 9a-H), 4.38–4.32 (1H, m, 5-H), 4.29 (1H, dd, $J_{6,5}$ = 8.0, $J_{6,7}$ = 4.0 Hz, 6-H), 4.17 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.2 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 3.73–3.59 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.32 (1H, br s, 4-H), 2.83–2.70 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.67–2.55 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.11 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 1.96 (3H, s, CH_3CONH); ^{13}C NMR ($CDCl_3$) δ 170.5, 170.2, 170.1, 170.0 (4C, 3XCH_3COO and $NHCOCH_3$), 161.8 (C-1), 144.6 (C-2), 108.7 (C-3), 77.2 (C-6), 71.0 (C-8), 68.2 (C-7), 67.1 (2C, N(CH2CH2)2O), 62.6 (C-4), 62.1 (C-9), 52.3 (COOCH3), 49.7 (2C, $N(CH_2CH_2)_2O$), 43.8 (C-5), 23.2 ($NHCOCH_3$), 20.8 (1C, CH_3COO), 20.7 (2C, 2XCH_3COO); MS (ESI positive) m/z 523.1 $[M + Na]^+$, 1022.5 $[2M + Na]^+$.

Preparation of methyl 5-acetamido-2,6-anhydro-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (7a)

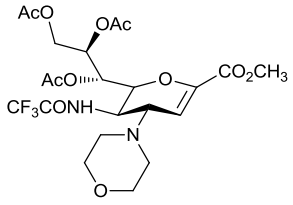


7a

The 2-hydroxy ester **5a** (103 mg, 0.20 mmol), dissolved in CH3CN (0.60 mL), is reacted with the TFAA (84 µL, 0.6 mmol) at 23 °C for 10 min. Then, the solvent and the TFAA excess are evaporated under a nitrogen flow to afford a crude residue recovered in CH_3CN (0.60 mL) and treated with TFA (46 µL, 0.6 mmol) at 135 °C for 5 min, in a sealed tube. Then, the reaction mixture is cooled, diluted with methanol (0.20 mL) and evaporated under reduced pressure to afford a crude product (90 mg) that was purified, by rapid chromatography, to afford the glycal **7a** (81 mg, 78%), as a white solid: m.p. 137–138 °C; $[\alpha]_D$ + 57.0 (c 1 in $CHCl_3$); (Found: C, 55.40; H, 6.51; N, 5.59; Calc. for: $C_{23}H_{34}N_2O_{10}$ C, 55.41; H, 6.87; N, 5.62%); ^1H NMR ($CDCl_3$) δ 6.06 (1H, d, $J_{3,4}$ = 3.0 Hz, 3-H), 5.61

(1H, d, $J_{NH,5}$ = 9.4 Hz, N–H), 5.50 (1H, dd, $J_{7,8}$ = $J_{7,6}$ = 3.7 Hz, 7-H), 5.30 (1H, ddd, $J_{8,9b}$ = 7.4, $J_{8,7}$ = 3.7, $J_{8,9a}$ = 3.3 Hz, 8-H), 4.64 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.3 Hz, 9a-H), 4.31–4.25 (1H, m, 5-H), 4.20 (1H, dd, $J_{6,5}$ = 8.6, $J_{6,7}$ = 3.7 Hz, 6-H), 4.14 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.4 Hz, 9b-H), 3.75 (3H, s, $COOCH_3$), 3.23 (1H, dd, $J_{4,5}$ = 8.1, J4,3 = 3.0 Hz, 4-H), 2.65–2.57 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.47–2.40 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.09 (3H, s, CH_3COO), 2.03–2.00 (6H, overlapping, $2XCH_3COO$), 1.89 (3H, s, CH_3CONH), 1.54–1.32 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.5, 170.2, 170.1, 170.0 (4C, $3XCH_3COO$ and $NHCOCH_3$), 162.0 (C-1), 144.2 (C-2), 110.7 (C-3), 77.4 (C-6), 71.3 (C-8), 68.2 (C-7), 63.1 (C-4), 62.1 (C-9), 52.2 ($COOCH_3$), 50.5 (2C, $N(CH_2CH_2)_2CH_2$), 43.9 (C-5), 26.4 (2C, $N(CH_2CH_2)_2CH_2$), 24.4 (1C, $N(CH_2CH_2)_2CH_2$), 23.3 ($NHCOCH_3$), 20.8 (2C, $2XCH_3COO$), 20.7 (1C, CH_3COO); MS (ESI positive) m/z 499.1 $[M + H]^+$, 521.1 $[M + Na]^+$, 1018.7 $[2M + Na]^+$.

Preparation of methyl 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-4-(morpholin- 4-yl) 7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate. (6b)

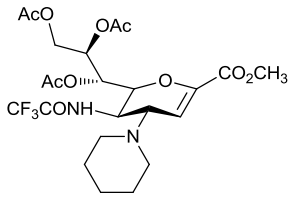


6b

Starting from the 2-hydroxy compound **4b** (114 mg, 0.20 mmol) the glycal **16b** (88 mg, 79%) is obtained, as a white solid: m.p. 138–140 °C; $[\alpha]_D$ + 84.9 (c 1 in $CHCl_3$); (Found: C, 47.99; H, 5.46; N, 5.11; Calc. for: $C_{22}H_{29}F_3N2O_{11}$ C, 47.66; H, 5.27; N, 5.05%); ^1H NMR ($CDCl_3$) δ 7.09 (1H, d, $J_{NH,5}$ = 9.6 Hz, N–H), 6.10 (1H, d, $J_{3,4}$ = 2.6 Hz, 3-H), 5.50 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 2.7 Hz, 7-H), 5.30–5.25 (1H, m, 8-H), 4.77 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 3.0 Hz, 9a-H), 4.34 (1H, dd, $J_{6,5}$ = 9.0, $J_{6,7}$ = 2.7 Hz, 6-H), 4.30–4.24 (1H, m, 5-H), 4.11 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 7.6 Hz, 9b-

H), 3.78 (3H, s, $COOCH_3$), 3.65–3.53 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.36 (1H, dd, $J_{4,5}$ = 9.1, $J_{4,3}$ = 2.6 Hz, 4-H), 2.74–2.67 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.45–2.43 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.09 (3H, s, CH_3COO), 2.02 (6H, overlapping, $2XCH_3COO$); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.5, 169.9 (3C, $3XCH_3COO$), 161.5 (C-1), 157.6 (q, $J_{C,F}$ = 37 Hz, $COCF_3$), 145.0 (C-2), 122.0–110.0 (1C, CF3), 108.4 (C-3), 76.6 (C-6), 71.3 (C-8), 68.1 (C-7), 67.1 (2C, $N(CH_2CH_2)_2O$), 62.6 (C-4), 62.0 (C-9), 52.4 ($COOCH_3$), 49.3 (2C, $N(CH_2CH_2)_2O$), 44.2 (C-5), 22.8 ($NHCOCH_3$), 20.7, 20.6, 20.5 (3C, $3XCH_3COO$); MS (ESI positive) m/z 555.8 $[M + H]^+$, 577.1 $[M + Na]^+$, 1131.5 $[2M + Na]^+$.

Preparation of methyl 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (7b)

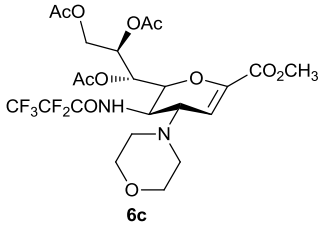


7b

Starting from the 2-hydroxy compound **5b** (114 mg, 0.20 mmol) the glycal **7b** (84 mg, 77%) is obtained, as a white solid: m.p. 123–125 °C; $[\alpha]_D$ + 89.5 (c 1 in $CHCl_3$); (Found: C, 50.26; H, 5.67; N, 4.98; Calc. for: $C_{23}H_{31}F_3N_2O_{10}$ C, 50.00; H, 5.66; N, 5.07%); ^1H NMR ($CDCl_3$) δ 6.96 (1H, br d, $J_{NH,5}$ = 8.7 Hz, N–H), 6.10 (1H, d, $J_{3,4}$ = 2.6 Hz, 3-H), 5.48 (1H, dd, $J_{7,8}$ = 4.1, $J_{7,6}$ = 2.6 Hz, 7-H), 5.28 (1H, ddd, $J_{8,9b}$ = 7.3, $J_{8,7}$ = 4.1, $J_{8,9a}$ = 3.1 Hz, 8-H), 4.74 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.1 Hz, 9a-H), 4.34–4.24 (2H, overlapping, 6-H and 5- H), 4.13 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.3 Hz, 9b-H), 3.78 (3H, s, $COOCH_3$), 3.36 (1H, dd, $J_{4,5}$ = 8.6, $J_{4,3}$ = 2.2 Hz, 4-H), 2.66–2.58 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.41–2.34 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.10 (3H, s, CH_3COO), 2.03 (6H, overlapping, $2XCH_3COO$), 1.54–1.34 (6H, overlapping, N $(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.9, 170.5, 170.0 (3C, $3XCH_3COO$), 161.7 (C-1), 157.7 (q, $J_{C,F}$ = 37 Hz, $COCF_3$), 144.5 (C-2), 120.0–110.0 (1C, CF_3), 110.1 (C-3), 76.7 (C-6), 71.4 (C-8),

68.1 (C-7), 63.1 (C-4), 62.1 (C-9), 52.3 ($COOCH_3$), 50.3 (2C, $N(CH_2CH_2)_2CH_2$), 44.6 (C-5), 26.3 (2C, N $(CH_2CH_2)_2CH_2$), 22.8 (1C, $N(CH_2CH_2)_2CH_2$), 20.7 (2C, $2XCH_3COO$), 20.5 (CH_3COO); MS (ESI positive) m/z 553.3 $[M + H]^+$, 575.1 $[M + Na]^+$, 1129.4 $[2M + Na]^+$.

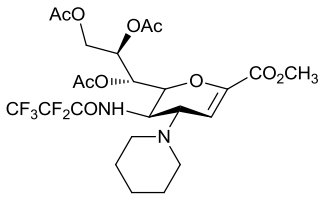
Preparation of methyl 2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (6c)



6c

Starting from the 2-hydroxy compound **4c** (124 mg, 0.20 mmol) the glycal **6c** (97 mg, 80%) is obtained, as a white solid: m.p. 128–130 °C; $[\alpha]_D$ + 78.2 (c 1 in $CHCl_3$); (Found: C, 45.98; H, 4.68; N, 4.69; Calc. for: $C_{23}H_{29}F_5N_2O_{11}$ C, 45.70; H, 4.84; N, 4.63%); ^1H NMR ($CDCl_3$) δ 6.95 (1H, d, $J_{NH,5}$ = 9.7 Hz, N–H), 6.13 (1H, d, $J_{3,4}$ = 2.5 Hz, 3-H), 5.48 (1H, dd, $J_{7,8}$ = 4.2, $J_{7,6}$ = 2.7 Hz, 7-H), 5.29 (1H, ddd, $J_{8,9b}$ = 7.3, $J_{8,7}$ = 4.2, $J_{8,9a}$ = 3.0 Hz, 8-H), 4.75 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.0 Hz, 9a-H), 4.37 (1H, dd, $J_{6,5}$ = 9.6, $J_{6,7}$ = 2.7 Hz, 6-H), 4.32–4.25 (1H, m, 5-H), 4.14 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.3 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 3.67–3.54 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.37 (1H, dd, $J_{4,5}$ = 9.3, $J_{4,3}$ = 2.5 Hz, 4-H), 2.75–2.68 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.47–2.41 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.10 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.9, 170.5, 169.9 (3C, $3XCH_3COO$), 161.5 (C-1), 158.3 (t, J_{C-F} = 26 Hz, $COCF_2CF_3$), 145.0 (C-2), 120.0–108.0 (2C, CF_2CF_3), 108.2 (C-3), 76.4 (C-6), 71.2 (C-8), 68.1 (C-7), 67.0 (2C, $N(CH_2CH_2)_2O$), 62.8 (C-4), 62.0 (C-9), 52.5 ($COOCH_3$), 49.2 (2C, $N(CH_2CH_2)_2O$), 44.4 (C-5), 20.8, 20.6, 20.5 (3C, 3XCH3COO); MS (ESI positive) m/z 605.2 $[M + H]^+$, 627.1 $[M + Na]^+$.

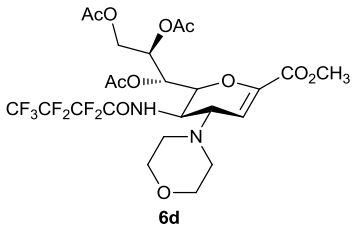
Preparation of methyl 2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (7c)



7c

Starting from the 2-hydroxy compound **5c** (124 mg, 0.20 mmol) the glycal **7c** (98 mg, 81%) was obtained, as a white solid: m.p. 140–143 °C; $[\alpha]_D$ + 59.7 (c 1 in $CHCl_3$); (Found: C, 47.92; H, 5.12; N, 4.56; Calc. for: $C_{24}H_{31}F_5N_2O_{10}$ C, 47.84; H, 5.19; N, 4.65%); ^{1}H NMR ($CDCl_3$) δ 6.88 (1H, br s, N–H), 6.12 (1H, d, $J_{3,4}$ = 2.5 Hz, 3- H), 5.46 (1H, dd, $J_{7,8}$ = 4.4, $J_{7,6}$ = 2.5 Hz, 7-H), 5.29 (1H, ddd, $J_{8,9b}$ = 7.2, $J_{8,7}$ = 4.4, $J_{8,9a}$ = 2.8 Hz, 8-H), 4.73 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.8 Hz, 9a-H), 4.35 (1H, dd, $J_{6,5}$ = 9.6, $J_{6,7}$ = 2.5 Hz, 6-H), 4.32–4.26 (1H, m, 5-H), 4.14 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.2 Hz, 9b-H), 3.79 (3H, s, $COOCH_3$), 3.37 (1H, dd, $J_{4,5}$ = 8.6, $J_{4,3}$ = 2.5 Hz, 4-H), 2.67–2.59 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.40–2.32 (2H, overlapping, $N(CH_2CH_2)_2$-CH_2), 2.11 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.55–1.35 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.4, 170.0 (3C, 3XCH_3COO), 161.7 (C-1), 158.2, (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_3$), 144.6 (C-2), 120.0–110.0 (2C, CF_2CF_3), 109.7 (C-3), 76.6 (C-6), 71.2 (C-8), 68.1 (C-7), 63.2 (C-4), 62.0 (C-9), 52.4 ($COOCH_3$), 50.3 (2C, $N(CH_2CH_2)_2CH_2$), 44.9 (C-5), 26.2 (2C, $N(CH_2CH_2)_2CH_2$), 24.4 (1C, $N(CH_2CH_2)_2CH_2$), 20.8, 20.6, 20.5 (3C, 3XCH_3COO); MS (ESI positive) m/z 603.2 $[M + H]^+$, 625.3 $[M + Na]^+$.

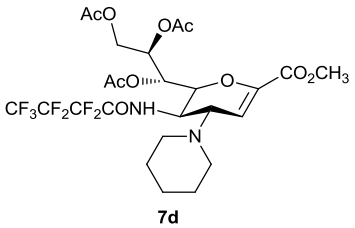
Preparation of methyl 2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (6d)



6d

Starting from the 2-hydroxy compound **4d** (135 mg, 0.20 mmol) the glycal **6d** (105 mg, 81%) is obtained, as a white solid: m.p. 127–129 °C; $[\alpha]^D$ + 77.7 (c 1 in $CHCl_3$); (Found: C, 44.15; H, 4.53; N, 4.20; Calc. for: $C_{24}H_{29}F_7N_2O_{11}$ C, 44.04; H, 4.47; N, 4.28%); ^{1}H NMR ($CDCl^3$) δ 6.97 (1H, d, $J_{NH,5}$ = 9.5 Hz, N–H), 6.13 (1H, d, $J_{3,4}$ = 2.4 Hz, 3-H), 5.48 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 2.8 Hz, 7-H), 5.32–5.27 (1H, m, 8-H), 4.73 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.0 Hz, 9a-H), 4.39 (1H, dd, $J_{6,5}$ = 9.5, $J_{6,7}$ = 2.8 Hz, 6-H), 4.31–4.22 (1H, m, 5-H), 4.15 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.2 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 3.67–3.52 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.40 (1H, dd, $J_{4,5}$ = 9.2, $J_{4,3}$ = 2.4 Hz, 4-H), 2.75–2.67 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.48–2.40 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.11 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.4, 170.0 (3C, 3XCH_3COO), 161.5 (C-1), 158.1 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_3$), 145.0 (C-2), 126.0–108.0 (3C, $CF_2CF_2CF_3$), 108.2 (C-3), 76.3 (C-6), 71.1 (C-8), 68.0 (C-7), 66.9 (2C, $N(CH_2CH_2)_2O$), 62.6 (C-4), 61.6 (C-9), 52.5 ($COOCH_3$), 49.2 (2C, $N(CH_2CH_2)_2O$), 44.6 (C-5), 20.8, 20.6, 20.5 (3C, 3XCH_3COO); MS (ESI positive) m/z 654.9 $[M + H]^+$, 677.1 $[M + Na]^+$, 1330.5 $[2M + Na]^+$.

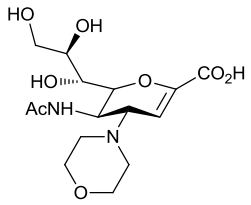
Preparation of methyl 2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (7d)



7d

Starting from the 2-hydroxy compound **5d** (134 mg, 0.20 mmol) the glycal **7d** (103 mg, 79%) was obtained, as a white solid: m.p. 137–139 °C; $[\alpha]_D$ + 93.0 (c 1 in $CHCl_3$); (Found: C, 45.94; H, 4.68; N, 4.33; Calc. for: $C_{25}H_{31}F_7N_2O_{10}$ C, 46.02; H, 4.79; N, 4.29%); ^{1}H NMR ($CDCl_3$) δ 6.90 (1H, br s, N–H), 6.12 (1H, d, $J_{3,4}$ = 2.6 Hz, 3- H), 5.45 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 2.6 Hz, 7-H), 5.30–5.26 (1H, m, 8-H), 4.73 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.9 Hz, 9a-H), 4.37 (1H, dd, $J_{6,5}$ = 9.0, $J_{6,7}$ = 2.6 Hz, 6-H), 4.28–4.21 (1H, m, 5-H), 4.14 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.2 Hz, 9b-H), 3.77 (3H, s, $COOCH_3$), 3.39 (1H, dd, $J_{4,5}$ = 9.1, $J_{4,3}$ = 2.6 Hz, 4-H), 2.68–2.57 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.39–2.31 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.10 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 2.02 (3H, s, CH_3COO), 1.53–1.34 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.2, 169.8 (3C, $3XCH_3COO$), 161.5 (C-1), 158.0, (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_3$), 144.4 (C-2), 120.0–110.0 (2C, $CF_2CF_2CF_3$), 109.5 (C-3), 76.4 (C-6), 71.1 (C-8), 67.9 (C-7), 63.0 (C-4), 61.8 (C-9), 52.1 ($COOCH_3$), 50.2 (2C, $N(CH_2CH_2)_2CH_2$), 44.7 (C-5), 26.0 (2C, $N(CH_2CH_2)_2CH_2$), 24.2 (1C, $N(CH_2CH_2)_2CH_2$), 20.6, 20.4, 20.3 (3C, $3XCH_3COO$); MS (ESI positive) m/z 653.1 $[M + H]^+$, 675.2 $[M + Na]^+$, 1326.6 $[2M + Na]^+$.

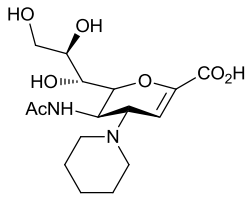
Preparation of 5-acetamido-2,6-anhydro-4-(morpholin-4-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (1a)



1a

The protected glycal **6a** (50 mg, 0.10 mmol), dissolved in aqueous methanol (1.0 mL, 1 : 2 v/v) saturated with K_2CO_4, is stirred for 12 h at 23 °C. Then the solution is treated with a acidic resin [DOWEX 50WX8 (H^+)] and stirred for 15 min. The solution is filtered and the solvent removed under reduced pressure to afford the free glycal **1a** (31 mg, 85%), as a white solid: m. p. 182–184 °C; $[\alpha]_D$ + 28.6 (c 1 in CH_3OH): (Found: C, 49.85; H, 6.76; N, 7.71; Calc. for: $C_{15}H_{24}N_2O_8$, C, 49.99; H, 6.71; N, 7.77%); ^1H NMR (D_2O) δ 5.77 (1H, d, $J_{3,4}$ = 2.8 Hz, 3-H), 4.36 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 9.4 Hz, 5-H), 4.17 (1H, d app, $J_{6,5}$ = 9.4 Hz, 6-H), 3.9 (1H, ddd, $J_{8,7}$ = 9.2, $J_{8,9b}$ = 6.2, $J_{8,9a}$ = 2.7 Hz, 8-H), 3.80 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.7 Hz, 9a-H), 3.79–3.80 (5H, overlapping, $N(CH_2CH_2)_2O$ and 4-H), 3.64 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.2 Hz, 9b-H), 3.60 (1H, dd, $J_{7,8}$ = 9.2, $J_{7,6}$ = 1.0Hz, 7-H), 2.81–2.74 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.70–2.64 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.04 (CH_3CONH); ^{13}C NMR (D_2O) δ 174.4 ($NHCOCH_3$), 168.9 (C-1), 151.2 (C-2), 101.2 (C-3), 75.8 (C-6), 69.9 (C-8), 68.5 (C-7), 66.3 (2C, $N(CH_2CH_2)_2O$), 63.1 (C-9), 62.7 (C-4), 48.6 (2C, $N(CH_2CH_2)_2O$), 42.8 (C-5), 22.3 ($NHCOCH_3$); MS (ESI negative) m/z 359.3 $[M - H]^-$.

Preparation of 5-acetamido-2,6-anhydro-4-(piperidin-1-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (7d)



2a

The protected glycal **7a** (50 mg, 0.10 mmol), dissolved in aqueous methanol (1.0 mL, 1 : 2 v/v) saturated with K_2CO_4, is stirred for 12 h at 23 °C. Then the solution is treated with a acidic resin [DOWEX 50WX8 (H^+)] and stirred for 15 min. The solution is filtered and the solvent removed under reduced pressure to afford the free glycal **2a** (32 mg, 91%) as a white solid: m. p. 166–168 °C; $[\alpha]_D$ + 24.3 (c 1 in CH_3OH); (Found: Calc. for: C, 53.63; H, 7.28; N, 7.80; $C_{16}H_{26}N_2O_7$ C, 53.62; H, 7.31; N, 7.82%); ^1H NMR (D_2O) δ 5.67 (1H, br s, 3-H), 4.51 (1H, t app., $J_{5,4} = J_{5,6} = 10.2$ Hz, 5-H), 4.23 (1H, d, $J_{6,5} = 10.2$ Hz, 6-H), 3.89–3.82 (1H, m, 8-H), 3.78 (1H, dd, $J_{9a,9b} = 12.0$, $J_{9a,8} = 1.8$ Hz, 9a-H), 3.61–3.45 (3H, overlapping, 4-H, 9b-H and 7-H), 3.43–3.20 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 3.10–2.74 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 1.85 (3H, s, CH_3CONH), 1.84–1.27 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR (D_2O) δ 175.7 ($NHCOCH_3$), 168.6 (C-1), 153.9 (C-2), 97.4 (C-3), 76.2 (C-6), 70.3 (C-8), 68.5 (C-7), 65.7 (C-9), 63.7 (C-4), 50.5 (2C, $N(CH_2CH_2)_2CH_2$), 43.0 (C-5), 23.9 (2C, N $(CH_2CH_2)_2CH_2$), 22.8 (1C, $N(CH_2CH_2)_2CH_2$), 21.9 (CH_3CONH); MS (ESI negative) m/z 357.1 $[M - H]^-$.

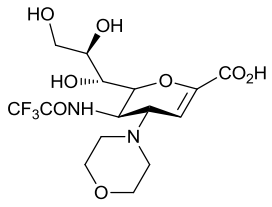
Selective hydrolysis of 4α-aminated N-perfluoracyl glycals 1b-d and 2b-d

General procedure

The appropriate protected glycal (0.1 mmol) dissolved in methanol–water (1.5 mL, 2 : 1 v/v) is treated with Et3N (0.90 mL) under stirring for 12 h at 23 °C. Then the solvent is removed under reduced pressure and the residue is

recovered with water and lyophilized many times until complete elimination of Et_3N.

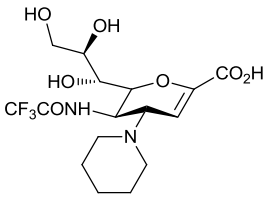
Preparation of 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-4-(morpholin-4-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (1b)



1b

Starting from the protected glycal **6b** (55 mg, 0.10 mmol) the free glycal **1b** (39 mg, 93%) is obtained, as a white solid: m. p. 187–189 °C; $[\alpha]_D$ + 28.6 (c 1 in CH_3OH); (Found: C, 43.54; H, 5.06; N, 6.68; Calc. for: $C_{15}H_{21}F_3N_2O_8$ C, 43.48; H, 5.11; N, 6.76%); ^{1}H NMR (D_2O) δ 5.84 (1H, d, $J_{3,4}$ = 2.2 Hz, 3-H), 4.50 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 10.0 Hz, 5-H), 4.33 (1H, d, $J_{6,5}$ = 10.0 Hz, 6-H), 3.96 (1H, ddd, $J_{8,7}$ = 9.3, $J_{8,9b}$ = 6.6, $J_{8,9a}$ = 2.6 Hz, 8-H), 3.89 (1H, dd, $J_{9a,9b}$ = 12.0, $J_{9a,8}$ = 2.6 Hz, 9a-H), 3.77–3.59 (6H, overlapping, $N(CH_2CH_2)_2$, 4-H and 9b-H), 3.56 (1H, d app, $J_{7,8}$ = 9.3 Hz, 7-H), 2.83–2.75 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.63–2.56 (2H, overlapping, $N(CH_2CH_2)_2O$); ^{13}CNMR (D_2O) δ 169.8 (C-1), 159.3 (q, $J_{C,F}$ = 37 Hz, $COCF_2$), 150.5 (C-2), 121.0–110.0 (1C, CF_3), 104.3 (C-3), 75.9 (C-6), 70.6 (C-8), 69.3 (C-7), 67.2 (2C, $N(CH_2CH_2)_2O$), 63.8 (C-9), 62.1 (C-4), 49.0 (2C, $N(CH_2CH_2)_2O$), 44.7 (C-5); MS (ESI negative) m/z 413.3 $[M - H]^-$.

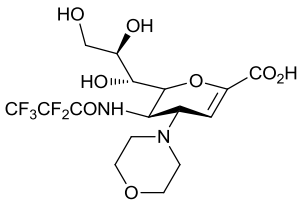
Preparation of 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-4-(piperidin-1-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (2b)



2b

Starting from compound **7b** (55 mg, 0.10 mmol) the free glycal **2b** is obtained (35 mg, 85%), as slightly yellow solid: m. p. 155–157 °C; $[\alpha]_D$ + 31.2 (c 1 in CH_3OH); (Found: C, 46.56; H, 5.58; N, 6.79; Calc. for: $C_{16}H_{23}F_3N_2O_7$ C, 46.60; H, 5.62; N, 6.79%); ^{1}H NMR (D_2O) δ 5.99 (1H, d, $J_{3,4}$ = 2.7 Hz, 3-H), 4.60 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 9.3 Hz, 5-H), 4.41 (1H, d app., $J_{6,5}$ = 9.3 Hz, 6-H), 3.91 (1H, ddd, $J_{8,7}$ = 9.0, $J_{8,9b}$ = 6.1, $J_{8,9a}$ = 2.2 Hz, 8-H), 3.87 (1H, dd, $J_{9a,9b}$ = 12.0, $J_{9a,8}$ = 2.2 Hz, 9a-H), 3.78 (1H, br s, 4-H), 3.66 (1H, dd, $J_{9b,9a}$ = 12.0, $J_{9b,8}$ = 6.1 Hz, 9b-H), 3.62 (1H, d, $J_{7,8}$ = 9.0 Hz, 7-H), 2.82–2.73 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.69–2.59 (2H, overlapping, $N(CH_2\text{-}CH_2)_2CH_2$), 1.64–1.45 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR (D_2O) δ 169.3 (C-1), 160.2 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_3$), 152.2 (C-2), 119.5–107.4 (2C, CF_2CF_3), 107.3 (C-3), 76.0 (C-6), 70.5 (C-8), 69.0 (C-7), 63.7 (C-9), 59.7 (C-4), 50.6 (2C, $N(CH_2CH_2)_2CH_2$), 44.0 (C-5), 24.7 (2C, $N(CH_2CH_2)_2CH_2$), 23.1 (1C, $N(CH_2CH_2)_2CH_2$); MS (ESI negative) m/z 411.3 $[M - H]^-$.

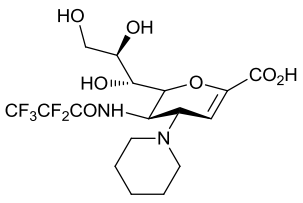
Preparation of 2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4-(morpholin-4-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (1c)



1c

Starting from the protected glycal **6c** (60 mg, 0.10 mmol) the free glycal **1c** (42 mg, 91%) was obtained, as a white solid: m. p. 144–146 °C; $[\alpha]_D$ + 26.4 (c 1 in CH_3OH); (Found: C, 41.25; H, 4.50; N, 6.00; Calc. for: $C_{16}H_{21}F_5N_2O_8$ C, 41.39; H, 4.56; N, 6.03%); ^{1}H NMR (D_2O) δ 5.84 (1H, d, $J_{3,4}$ = 2.2 Hz, 3-H), 4.75 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 9.6 Hz, 5-H), 4.44 (1H, d, $J_{6,5}$ = 9.6 Hz, 6-H), 4.40 (1H, dd, $J_{4,5}$ = 9.6, $J_{4,3}$ = 2.4 Hz, 4-H), 4.04–3.96 (2H, overlapping, $N(CH_2CH_2)_2O$), 3.96–3.88 (3H, overlapping, $N(CH_2CH_2)_2O$ and 8-H), 3.84 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.5 Hz, 9a-H), 3.58 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.3 Hz 9b-H), 3.56–3.48 (3H, overlapping, $N(CH_2CH_2)_2O$ and 7-H), 3.29–3.21 (2H, overlapping, $N(CH_2CH_2)_2O$); ^{13}C NMR (D_2O) δ 167.5 (C-1), 160.0 (t, $J_{C,F}$ = 26 Hz, $COCF_2$), 153.3 (C-2), 119.5–107.4 (2C, CF_2CF_3), 96.6 (C-3), 75.4 (C-6), 69.7 (C-8), 68.2 (C-7), 63.9 (3C, $N(CH_2CH_2)_2O$ and C-9), 63.0 (C-4), 48.7 (2C, $N(CH_2CH_2)2O$), 43.2 (C-5); MS (ESI negative) m/z 463.2 $[M - H]^-$.

Preparation of 2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4-(piperidin-1-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (2c)

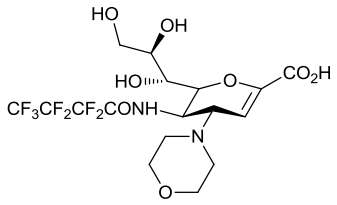


2c

Starting from the protected glycal **7c** (60 mg, 0.10 mmol) the free glycal **2c** (39 mg, 84%) is obtained, as slightly yellow solid: m.p. 155–157 °C; $[\alpha]_D$ + 22.3 (c 1

in CH_3OH); (Found: C, 44.09; H, 4.96; N, 6.03; Calc. for: $C_{17}H_{23}F5N_2O_7$ C, 44.16; H, 5.01; N, 6.06%); ^{1}H NMR (D_2O) δ 5.80 (1H, d, $J_{3,4}$ = 2.5 Hz, 3-H), 4.73 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 10.0 Hz, 5-H), 4.40 (1H, d app, $J_{6,5}$ = 10.0 Hz, 6-H), 4.22 (1H, br s, 4-H), 3.96 (1H, ddd, $J_{8,7}$ = 9.3, J_{8-9b} = 6.3, $J_{8,9a}$ = 2.4 Hz, 8-H), 3.89 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.4 Hz, 9a-H), 3.62 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.3 Hz, 9b-H), 3.54 (1H, d app., $J_{7,8}$ = 9.3 Hz, 7-H), 3.33–3.19 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 3.09–2.96 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 1.81–1.55 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR (D_2O) δ 168.3 (C-1), 159.8 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_3$), 152.4 (C-2), 119.5–107.4 (2C, CF_2CF_3), 99.3 (C-3), 75.4 (C-6), 69.8 (C-8), 68.3 (C-7), 63.3 (C-9), 63.1 (C-4), 50.2 (2C, $N(CH_2CH_2)_2CH_2$), 43.3 (C-5), 23.7 (2C, $N(CH_2CH_2)_2CH_2$), 22.0 (1C, $N(CH_2CH_2)_2CH_2$); MS (ESI negative) m/z 461.1 $[M - H]^-$.

Preparation of 2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(morpholin-4-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (1d)

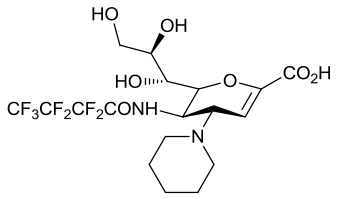


1d

Starting from the protected glycal **6d** (65 mg, 0.10 mmol) the free glycal **1d** (48 mg, 94%) is obtained, as a white solid: m. p. 148–151 °C; $[\alpha]_D$ + 25.7 (c 1 in CH_3OH); (Found: C, 39.65; H, 4.09; N, 5.50; Calc. for: $C_{17}H_{21}F_7N_2O_8$ C, 39.70; H, 4.12; N, 5.45%); ^{1}H NMR (D_2O) δ 5.84 (1H, d, $J_{3,4}$ = 2.4 Hz, 3-H), 4.52 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 9.9 Hz, 5-H), 4.34 (1H, d, $J_{6,5}$ = 9.9 Hz, 6-H), 3.95 (1H, ddd, $J_{8,7}$ = 9.4, $J_{8,9b}$ = 6.6, $J_{8,9a}$ = 2.5 Hz, 8-H), 3.89 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.5 Hz, 9a-H), 3.79–3.68 (5H, overlapping, $N(CH_2CH_2)_2O$ and 4-H), 3.61 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.6 Hz, 9b-H), 3.56 (1H, d, $J_{7,8}$ = 9.4 Hz, 7-H), 2.88–2.81 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.69–2.62 (2H, overlapping, $N(CH_2CH_2)_2O$); ^{13}C NMR (D_2O) δ 169.8 (C-1), 159.9 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_2$), 150.6 (C-2), 120.0–111.5 (3C,

$CF_2CF_2CF_3$), 104.0 (C-3), 75.8 (C-6), 70.6 (C-8), 69.3 (C-7), 67.2 (2C, $N(CH_2CH_2)_2O$), 63.8 (C-9), 62.4 (C-4), 49.0 (2C, $N(CH_2CH_2)_2O$), 45.0 (C-5); MS (ESI negative) m/z 513.2 $[M - H]^-$.

Preparation of 2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(piperidin-1-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (2d)

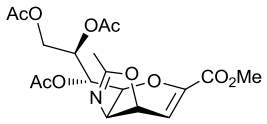


2d

Starting from the protected glycal **7d** (65 mg, 0.10 mmol) the free glycal **2d** (44 mg, 85%) is obtained, as a white solid: m.p. 188–190 °C; $[\alpha]_D$ + 24.9 (c 1 in CH_3OH); (Found: C, 42.10; H, 4.50; N, 5.43; Calc. for: $C_{18}H_{23}F_7N_2O_7$ C, 42.19; H, 4.52; N, 5.47%); ^1H NMR (D_2O) δ 5.80 (1H, d, $J_{3,4}$ = 1.6 Hz, 3-H), 4.74 (1H, under water signal, 5-H), 4.40 (1H, d app, $J_{6,5}$ = 10.0 Hz, 6-H), 4.22 (1H, br s, 4-H), 3.96 (1H, m, 8-H), 3.88 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 1.8 Hz, 9a-H), 3.62 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.4 Hz, 9b-H), 3.55 (1H, d app., $J_{7,8}$ = 9.2 Hz, 7- H), 3.30–3.23 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 3.23–3.15 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 1.93–1.47 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR (D_2O) δ 168.3 (C-1), 159.8 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_3$), 152.4 (C-2), 119.5–107.4 (3C, $CF_2CF_2CF_3$), 99.3 (C-3), 75.4 (C-6), 69.8 (C-8), 68.3 (C-7), 63.3 (C-9), 63.1 (C-4), 50.2 (2C, $N(CH_2CH_2)_2CH_2$), 43.3 (C-5), 23.7 (2C, $N(CH_2CH_2)_2CH2$), 22.0 (1C, $N(CH_2CH_2)_2CH_2$); MS (ESI negative) m/z 511.2 $[M - H]^-$.

5.3.2 Synthesis of C-4β hydroxy derivatives of normal and *N*-perfluorinated Neu5Ac2en

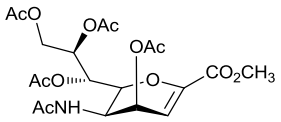
Preparation of Methyl 7,8,9-tri-*O*-acetyl-2,3,4,5-tetradeoxy-2,3-didehydro-2,3-trideoxy-4′,5′-dihydro-2′-methyloxazolo-[5,4]-D-*glycero*-D-*talo*-non-2-enoate (16):



16

Starting from compound **3** (95 mg, 0.2 mmol) and performing the reaction at 80 °C for 5 min in MeCN (Entry 2), with H_2SO_4 (2 mmol) and Ac_2O (2 mmol) the oxazoline **16** (78 mg, 95%) was obtained as a glass: $[\alpha]_D$ – 60.0 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$) δ = 6.38 (1H, d, $J_{3,4}$ = 4.0 Hz, 3-H), 5.62 (1H, dd, $J_{7,6}$ = 2.6, $J_{7,8}$ = 5.9 Hz, 7-H), 5.43 (1H, ddd, $J_{8\text{-}9a}$ = 2.6, $J_{8,7}$ = 5.9, $J_{8,9b}$ = 6.3 Hz, 8-H), 4.81 (1H, dd, $J_{4,3}$ = 4.0, $J_{4,5}$ = 8.6, 4-H), 4.59 (1H , dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.22 (1H, dd, $J_{9b,8}$ = 6.3, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.95 (1H, dd, $J_{5,4}$ = 8.6, $J_{5,6}$ = 10.5 Hz, 5-H), 3.80 (3H, s, $COOCH_3$), 3.42 (1H, dd, $J_{6,7}$ = 2.6, $J_{6,5}$ = 10.5 Hz, 6-H), 2.14 (3H, s, $OCOCH_3$), 2.04 (6H, overlapping, 2 X $OCOCH_3$), 2.00 (3H, s, CCH_3) ppm. ^{13}C NMR ($CDCl_3$) δ = 170.6, 169.8, 169.6 (3C, CH_3COO), 167.2 (CCH_3), 161.8 (C-1), 147.0 (C-2), 107.5 (C-3), 76.6 (C-6), 72.2 (C-4), 70.2 (C-8), 68.7 (C-7), 61.9 (C-9), 61.8 (C-5), 52.4 ($COOCH_3$), 20.7, 20.6, 20.5 (3C, CH_3COO), 14.0 (1C, CCH_3) ppm. MS (ESI positive) *m/z* 414.5 $[M+H]^+$, 437.4 $[M+Na]^+$. $C_{18}H_{23}NO_{10}$: calcd C, 52.30; H, 5.61; N, 3.39; found C, 52.24; H, 5.49; N, 3.33.

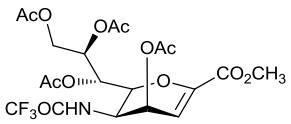
Preparation of methyl 2,6-anhydro-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (13):



13

A solution of compound **3** (95 mg, 0.2 mmol) in MeCN (1.5 mL) containing H_2SO_4 (106 µL, 2.0 mmol) and Ac_2O (471 µL, 5.0 mmol) is heated at 80 °C for 5 min. Then Et_3N (335 µL, 2.4 mmol) and H_2O (360 µL, 20.0 mmol) are added and the solution is stirred for 10 min, at 23°C. Usual work-up and rapid chromatography, eluting with AcOEt, afford in the glycal **13** (88 mg, 92%) ^{1}H NMR ($CDCl_3$): δ = 6.15 (1H, d, $J_{3,4}$ = 5.4 Hz, 3-H), 5.84 (1H, d, $J_{NH,5}$ = 10.1 Hz, N-H), 5.45 (1H, dd, $J_{7,6}$ = 2.1, $J_{7,8}$ = 3.8 Hz, 7-H), 5.26 (1H, ddd, $J_{8,9a}$ = 2.7, $J_{8,7}$ = 3.8, $J_{8\text{-}9b}$ = 7.3Hz, 8-H), 5.11 (1H, dd, $J_{4,5}$ = 4.6, $J_{4,3}$ = 5.4 Hz, 4-H), 4.73 (1H, dd, $J_{9a,8}$ = 2.7, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.53 (1H, ddd, $J_{5,4}$ = 4.6, $J_{5,6}$ = $J_{5,NH}$ = 10.1 Hz, 5-H), 4.24 (1H, dd, $J_{6,7}$ = 2.1, $J_{6,5}$ = 10.1 Hz, 6-H), 4.14 (1H, dd, $J_{9b,8}$ = 7.3, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.75 (3H, s, $COOCH_3$), 2.05 (6H, overlapping, 2 X CH_3COO), 2.03 (3H, s, $COOCH_3$), 2.01 (3H, s, $COOCH_3$), 1.88 (3H, s, CH_3CONH) ppm. $C_{20}H_{27}NO_{12}$: calcd C, 50.74; H, 5.75; N, 2.96; found C, 50.69; H, 5.67; N, 3.03. All these physico-chemical properties are in agreement with those reported.

Preparation of methyl 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (14b).

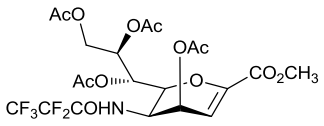


14b

By *N*-transacylation of **13**: the 4β-acetoxy glycal **13** (200 mg, 0.42 mmol) is directly *N*-transacylated with trifluoroacetic anhydride according to our procedure[13] to afford the fluorinated compound **14b** as a white solid (186 mg, 84%): m.p. 141-143°C; $[\alpha]_D$ - 41.2 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 6.76 (d,

$J_{NH,5}$ = 10.1 Hz, 1H, N-H), 6.21 (d, $J_{3,4}$ = 5.5 Hz, 1H, 3-H), 5.45 (dd, $J_{7,6}$ = 2.5, $J_{7,8}$ = 4.4 Hz, 1H, 7-H), 5.30 (ddd, $J_{8,9a}$ = 2.9, $J_{8,7}$ = 4.4, $J_{8,9b}$ = 7.2 Hz, 1H, 8-H), 5.26 (dd, $J_{4,5}$ = 4.2, $J_{4,3}$ = 5.5 Hz, 1H, 4-H), 4.72 (dd, $J_{9a,8}$ = 2.9, $J_{9a,9b}$ = 12.4 Hz, 1H, 9a-H), 4.53 (ddd, $J_{5,4}$ = 4.2, $J_{5,6}$ = $J_{5,NH}$ = 10.1 Hz, 1H, 5-H), 4.39 (dd, $J_{6,7}$ = 2.5, $J_{6,5}$ = 10.1 Hz, 1H, 6-H), 4.17 (dd, $J_{9b,8}$ = 7.2, $J_{9b,9a}$ = 12.4 Hz, 1H, 9b-H), 3.80 (s, 3H, $COOCH_3$), 2.10 (s, 3H, CH_3COO), 2.09 (s, 3H, CH_3COO), 2.07 (s, 3H, CH_3COO), 2.04 (s, 3H, CH_3COO). ^{13}C NMR ($CDCl_3$): δ = 170.6, 170.4, 169.7, 169.5 (4C, CH_3COO), 161.3 (C-1), 157.5-156.5 (q, J_{C-F} = 38 Hz, $COCF_3$), 146.4 (C-2), 118.8-111.9 (q, J_{C-F} = 287.6 Hz, $COCF_3$), 105.5 (C-3), 73.3 (C-6), 71.3 (C-8), 67.5 (C-7), 63.9 (C-4), 61.9 (C-9), 52.6 ($COOCH_3$), 45.3 (C-5), 20.8, 20.7, 20.6, 20.4 (4C, CH_3COO). MS (ESI positive) *m/z* 528.3 $[M+H]^+$, 550.2 $[M+Na]^+$, 1077.5 $[2M+Na]^+$. $C_{20}H_{24}F_3NO_{12}$: calcd C, 45.55; H, 4.59; N, 2.66; found C, 45.75; H, 4.41; N, 2.55.

Preparation of methyl 2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (14c)

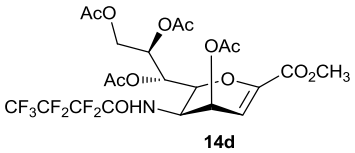


14c

4β-Acetoxy glycal **13** (200 mg, 0.42 mmol), obtained as above reported, is directly *N*-transacylated with pentafluoropropionic anhydride according to our procedure[15] [81] to afford the fluorinated compound **14c** as a white solid (209 mg, 86%): m.p. 149-151°C; $[\alpha]_D$ – 53.8 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.85 (1H, d, $J_{NH,5}$ = 9.7 Hz, N-H), 6.20 (1H, d, $J_{3,4}$ = 5.4 Hz, 3-H), 5.44 (1H, dd, $J_{7,6}$ = 2.5, $J_{7,8}$ = 4.4 Hz, 7-H), 5.32-5.27 (2H, overlapping, 8-H and 4-H), 4.69 (1H, dd, $J_{9a,8}$ = 2.8, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.55 (1H, ddd, $J_{5,4}$ = 4.4, $J_{5,NH}$ = 9.7, $J_{5,6}$ = 10.3 Hz, 5-H), 4.39 (1H, dd, $J_{6,7}$ = 2.5, $J_{6,5}$ = 10.3 Hz, 6-H), 4.16 (1H, dd, $J_{9b,8}$ = 7.0, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 2.09 (6H, overlapping, 2 X CH_3COO), 2.07 (3H, s, $COOCH_3$), 2.04 (3H, s, $COOCH_3$) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.6, 170.4, 169.6, 169.4(4C, CH_3COO), 161.4 (C-1), 157.6 (t, J_{C-F} = 26 Hz, $COCF_2$),

146.2 (C-2), 119.0-110.0 (2C, CF_2CF_3), 105.6 (C-3), 73.2 (C-6), 71.2 (C-8), 67.4 (C-7), 63.6 (C-4), 61.9 (C-9), 52.7 ($COOCH_3$), 45.2 (C-5), 20.8, 20.6, 20.5, 20.4 (4C, CH_3COO) ppm. MS (ESI positive) *m/z* 578.4 $[M+H]^+$, 600.3 $[M+Na]^+$, 1177.9 $[2M+Na]^+$. $C_{21}H_{24}F_5NO_{12}$: calcd C, 43.68; H, 4.19; N, 2.43; found C, 44.66; H, 4.83; N, 2.74.

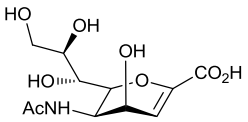
Preparation of methyl 2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (14d)



14d

The 4β-acetoxy glycal **13** (200 mg, 0.42 mmol), obtained as above reported, is directly *N*-transacylated with heptafluorobutirric anhydride according to our procedure[81] to afford the fluorinated compound **14d** as a white solid (216 mg, 82%): m.p. 133-135°C; $[\alpha]_D$ – 62.5 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.62 (1H, br s, N-H), 6.22 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.46 (1H, dd, $J_{7,6}$ = 2.6, $J_{7,8}$ = 4.5 Hz, 7-H), 5.32 (1H, ddd, $J_{8,9a}$ = 2.9, $J_{8,7}$ = 4.5, $J_{8\text{-}9b}$ = 6.8 Hz, 8-H), 5.28 (1H, dd, $J_{4,5}$ = 4.3, $J_{4,3}$ = 5.3 Hz, 4-H), 4.70 (1H, dd, $J_{9a,8}$ = 2.9, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.60 (1H, ddd, $J_{5,4}$ = 4.3, $J_{5,6}$ = $J_{5,NH}$ = 10.2 Hz, 5-H), 4.39 (1H, dd, $J_{6,7}$ = 2.6, $J_{6,5}$ = 10.2 Hz, 6-H), 4.18 (1H, dd, $J_{9b,8}$ = 6.8, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.81 (3H, s, $COOCH_3$), 2.11-2.08 (9H, overlapping, 3 X CH_3COO), 2.06 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.4, 169.6, 169.2 (4C, CH_3COO), 161.3 (C-1), 157.4 (t, $J_{C\text{-}F}$ = 26 Hz, $COCF_2$), 146.3 (C-2), 119.0-110.0 (3C, $CF_2CF_2CF_3$), 105.6 (C-3), 73.3 (C-6), 71.3 (C-8), 67.4 (C-7), 63.7 (C-4), 61.9 (C-9), 52.7 ($COOCH_3$), 45.4 (C-5), 20.8, 20.7, 20.5, 20.4 (4C, CH_3COO) ppm. MS (ESI positive) *m/z* 628.3 $[M+H]^+$, 650.5 $[M+Na]^+$. $C_{22}H_{24}F_7NO_{12}$: calcd C, 42.11; H, 3.86; N, 2.23; found C, 44.03; H, 3.51; N, 1.98.

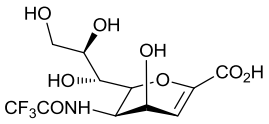
Preparation of 2,6-anhydro-5-acetamido-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enoic acid (11a):



11a

The protected glycal **13** (50 mg, 0.10 mmol), dissolved in aqueous methanol (1.0 mL, 1 : 2 v/v) saturated with K_2CO_4, is stirred for 12 h at 23 °C. Then the solution is treated with a acidic resin [DOWEX 50WX8 (H^+)] and stirred for 15 min. The solution is filtered and the solvent removed under reduced pressure to afford the free glycal **11a** (31 mg, 85%), as a white solid: Calcd for: $C_{11}H_{17}NO_8$ C, 45.36; H, 5.88; N, 4.81%; found C, 45.01; H, 5.17; N, 4.33); ^{1}H NMR (MeOD) δ = 5.86 (1H, d, $J_{3,4}$ = 5.8 Hz, 3-H), 4.28-4.16 (3H, overlapping, 4-H, 6-H and 5-H), 3.98 (1H, ddd, $J_{8,9a}$ = 2.6, $J_{8,9b}$ = 6.5, $J_{8,7}$ = 9.3 Hz, 8-H), 3.90 (1H, dd, $J_{9a,8}$ = 2.6, $J_{9,-9b}$ = 11.4 Hz, 9a-H), 3.66 (1H, dd, $J_{9b,8}$ = 6.5, $J_{9b,9a}$ = 11.9 Hz, 9b-H), 3.62 (1H, d app, $J_{7,8}$ = 9.3 Hz, 7-H); ^{13}C NMR (D_2O) δ = 174.2 (C-1), 169.8 ($NHCOCH_3$), 149.1 (C-2), 104.6 (C-3), 70.9 (C-6), 69.9 (C-8), 68.4 (C-7), 63.2 (C-9), 60.8 (C-5), 47.7 (C-4), 21.9 ($NHCOCH_3$). MS (ESI negative) *m/z* 290.1 $[M-H]^-$.

Preparation of 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enoic acid (11b):

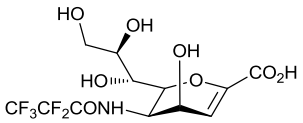


11b

the title compound **11b** is prepared by selective hydrolysis of glycal 1**4b** (72 mg, 0.14 mmol) performed in a solution of methanol-water (1.5 mL, 2:1 v/v) containing Et_3N (0.90 mL), kept a 23°C for 12 h. Then the solvent is removed under reduced pressure and the residue recovered with aqueous methanol and lyophilized, to afford glycal **11b** as a white solid (48 mg, 90%): m.p. 112-

114°C (from MeOH,-diisopropylether); $[\alpha]_D$ - 23.2 (*c* 1 in MeOH). Calcd for: $C_{11}H_{14}F_3NO_8$ C, 38.27; H, 4.09; N, 4.06%; found: C, 37.83; H, 3.89; N, 3.84); ^{1}H NMR (D_2O) δ = 5.89 (1H, d, $J_{3,4}$ = 5.6 Hz, 3-H), 4.39-4.30 (13H, overlapping, 6-H, 4H and 5-H), 4.00 (1H, ddd, $J_{8,9a}$ = 2.5, $J_{8,9b}$ = 6.4, $J_{8,7}$ = 9.3 Hz, 8-H), 3.91 (1H, dd, $J_{9a,8}$ = 2.7, $J_{9a,9b}$ = 11.9 Hz, 9a-H), 3.65 (1H, dd, $J_{9b,8}$ = 6.4, $J_{9b,9a}$ = 11.9 Hz, 9b-H), 3.60 (1H, d app, $J_{7,8}$ = 9.3 Hz, 7-H); ^{13}C NMR (D_2O) δ = 170.2 (C-1), 159.1 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 149.9 (C-2), 120.0-110.0 (1C, CF_3), 105.1 (C-3), 71.1 (C-6), 70.7 (C-8), 69.0 (C-7), 63.8 (C-9), 61.2 (C-5), 49.3 (C-4). MS (ESI negative) *m/z* 344.2 $[M-H]^-$.

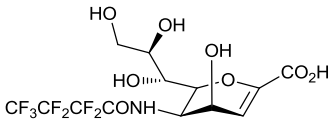
Preparation of 2,6-anhydro-5--(2,2,3,3,3-pentafluoropropionamido)-3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enoic acid (11c):



11C

the title compound **11c** is prepared by selective hydrolysis of glycal 1**4c** (80 mg, 0.14 mmol) performed in a solution of methanol-water (1.5 mL, 2:1 v/v) containing Et_3N (0.90 mL), kept a 23°C for 12 h. Then the solvent is removed under reduced pressure and the residue recovered with aqueous methanol and lyophilized, to afford glycal **11b** as a white solid (49 mg, 91%): m.p. 107-109°C (from MeOH,-diisopropylether); $[\alpha]_D$ – 35.1 (*c* 1 in MeOH). Calcd for: $C_{12}H_{14}F_5NO_8$ C, 36.47; H, 3.57; N, 3.54%; found: C, 36.04; H, 3.28; N, 3.33); ^{1}H NMR (D_2O) δ = 5.89 (1H, d, $J_{3,4}$ = 5.9 Hz, 3-H), 4.41-4.37 (2H, overlapping, 6-H and 5-H), 4.31 (1H, dd, $J_{4,5}$ = 1.1, $J_{4,3}$ = 5.8 Hz, 4-H), 4.00 (1H, ddd, $J_{8\text{-}9a}$ = 2.5, $J_{8,9b}$ = 6.5, $J_{8,7}$ = 9.2 Hz, 8-H), 3.90 (1H, dd, $J_{9a,8}$ = 2.5, $J_{9a,9b}$ = 11.9 Hz, 9a-H), 3.65 (1H, dd, $J_{9b,8}$ = 6.5, $J_{9b,9a}$ = 11.9 Hz, 9b-H), 3.57 (1H, d app, $J_{7,8}$ = 9.5 Hz, 7-H); ^{13}C NMR (MeOD) δ = 170.1 (C-1), 159.7 (t, $J_{C\text{-}F}$ = 26 Hz, $\underline{C}OCF_2$), 149.8 (C-2), 120.0-108.2 (2C, $CF2CF_3$), 104.1 (C-3), 70.9 (C-6), 70.6 (C-8), 69.0 (C-7), 63.7 (C-9), 61.1 (C-5), 49.4 (C-4). MS (ESI negative) *m/z* 394.2 $[M-H]^-$.

Preparation of 2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enoic acid (11d):

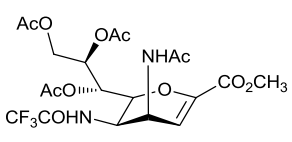


11d

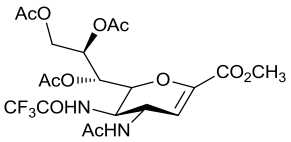
the title compound **11d** is prepared by selective hydrolysis of glycal 1**4d** (85 mg, 0.14 mmol) performed in a solution of methanol-water (1.5 mL, 2:1 v/v) containing Et_3N (0.90 mL), kept a 23°C for 12 h. Then the solvent is removed under reduced pressure and the residue recovered with aqueous methanol and lyophilized, to afford glycal **11b** as a white solid (49 mg, 91%): m.p. 107-109°C (from MeOH,-diisopropylether); $[\alpha]_D$ – 35.1 (*c* 1 in MeOH). Calcd for: $C_{13}H_{14}F_7NO_8$ C, 35.07; H, 3.17; N, 3.15%; found: C, 34.15; H, 2.98; N,3.01); ^{1}H NMR (MeOD) δ = 5.84 (1H, d, $J_{3,4}$ = 5.7 Hz, 3-H), 4.43 (1H, d app, $J_{6,5}$ = 11.5 Hz, 6-H), 4.29 (1H, dd, $J_{5,4}$ = 3.8, $J_{5,6}$ = 11.5 Hz, 5-H), 4.18 (1H, dd, $J_{4,5}$ = 3.8, $J_{4,3}$ = 5.7 Hz, 4-H), 3.90 (1H, ddd, $J_{8,9a}$ = 2.7, $J_{8,9b}$ = 5.7, $J_{8,7}$ = 8.9 Hz, 8-H), 3.85 (1H, dd, $J_{9a\text{-}8}$ = 2.7, $J_{9a,9b}$ = 11.4 Hz, 9a-H), 3.63 (1H, dd, $J_{9b,8}$ = 5.7, $J_{9b,9a}$ = 11.4 Hz, 9b-H), 3.50 (1H, d app, $J_{7,8}$ = 8.9 Hz, 7-H); ^{13}C NMR (MeOD) δ = 170.3 (C-1), 159.1 (t, $J_{C\text{-}F}$ = 26 Hz, $\underline{C}OCF_2$), 151.3 (C-2), 124.0-108.2 (3C, CF_2CF2CF_3), 104.9 (C-3), 71.9 (C-6), 71.7 (C-8), 70.3 (C-7), 65.1 (C-9), 61.9 (C-5), 50.4 (C-4). MS (ESI negative) *m/z* 444.2 $[M\text{-}H]^-$.

5.3.3 Synthesis of C-4β *N*-acetamido derivatives of normal and *N*-perfluorinated Neu5Ac2en

Preparation of methyl 4-acetamido-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-glycero-D-talo-non-2-enonate (20) and methyl 4-acetamido-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonate (21)



20



21

Starting from glycal **19** (105 mg, 0.2 mmol), after a 5 min heating at 80 °C in MeCN a mixture of the glycals **20** and **21** is obtained that, after rapid chromatography, eluting with AcOEt/hexane (80:20 v/v), affords in the sequence, the less polar glycal **21** (8 mg, 8%) followed by the more polar glycal **20** (72 mg, 72%), both in pure form. Glycal **21** showed: m.p. 133-135°C (from CH_2Cl_2-diisopropylether); $[\alpha]_D$ + 41.3 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 8.14 (d, $J_{NH,5}$ = 9.9 Hz, 1H, CF_3CONH), 5.93 (d, $J_{NH,4}$ = 9.7 Hz, 1H, CH_3CONH), 5.88 (d, $J_{3,4}$ = 2.0 Hz, 1H, 3-H), 5.48 (dd, $J_{7,6}$ = 1.4, $J_{7,8}$ = 4.4 Hz, 1H, 7-H), 5.31 (m, 1H, 8-H), 5.03 (ddd, $J_{4,3}$ = 2.0, $J_{4,5}$ = $J_{4,NH}$ = 9.7 Hz,1H, 4-H), 4.59 (dd, $J_{9a,8}$ = 2.5, $J_{9a,9b}$ = 12.4 Hz, 1H, 9a-H), 4.49 (dd, $J_{6,7}$ = 1.4, $J_{6,5}$ = 9.8 Hz, 1H, 6-H), 4.24-4.15 (overlapping, 2H, 9b-H and 5-H), 3.80 (s, 3H, $COOCH_3$), 2.09 (s, 3H, CH_3COO), 2.07 (s, 3H, CH_3COO), 2.05 (s, 3H, CH_3COO), 1.97 (s, 3H, CH_3CONH) ppm.^{13}C NMR ($CDCl_3$): δ = 171.3 (CH_3CONH), 170.5, 170.1, 169.4 (3C, CH_3COO), 161.5 (C-1), 158.8-157.8 (q, J_{C-F} = 38 Hz, $COCF_3$), 144.8 (C-2), 119.0-112.2 (1C, CF_3), 110.2 (C-3), 76.4 (C-6), 70.6 (C-8), 67.8 (C-7), 62.1 (C-9), 52.4 ($COOCH_3$), 48.0 (C-5), 47.7 (C-4), 22.6 (CH_3CONH), 20.7, 20.5, 20.3 (3C, CH_3COO) ppm. MS (ESI positive) *m/z* 527.3 $[M+H]^+$, 549.1 $[M+Na]^+$, 1075.5 $[2M+Na]^+$. $C_{20}H_{25}F_3N_2O_{11}$: calcd. C, 45.63; H, 4.79; N, 5.32; found C, 45.56; H, 4.67; N, 5.30.

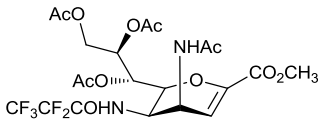
Glycal **20** showed: m.p. 146-148 °C (from CH_2Cl_2-diisopropylether); $[\alpha]_D^{20}$ -20.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.78 (d, $J_{NH,5}$ = 9.2 Hz, 1H, CF_3CONH), 6.59 (d, $J_{NH,4}$ = 8.7 Hz, 1H, CH_3CONH), 5.98 (d, $J_{3,4}$ = 5.1 Hz, 1H, 3-H), 5.49 (dd, $J_{7,6} = J_{7,8}$ = 3.3 Hz, 1H, 7-H), 5.27 (ddd, $J_{8,9a} = J_{8,7}$ = 3.2, $J_{8,9b}$ = 8.2 Hz, 1H, 8-H), 4.90 (dd, $J_{4,5} = J_{4,3}$ = 5.1 Hz, $J_{4,NH}$ = 8.7 Hz, 4-H), 4.81 (dd, $J_{9a,8}$ = 3.2, $J_{9a,9b}$ = 12.3 Hz, 1H, 9a-H), 4.38 (ddd, $J_{5,4}$ = 5.1, $J_{5,6} = J_{5,NH}$ = 9.2 Hz, 1H, 5-H), 4.30 (dd, $J_{6,7}$ = 3.3, $J_{6,5}$ = 9.2 Hz, 1H, 6-H), 4.15 (dd, $J_{9b,8}$ = 8.2, $J_{9b,9a}$ = 12.3 Hz, 1H, 9b-H), 3.78 (s, 3H, $COOCH_3$), 2.05-2.01 (overlapping, 9H, 3XCH_3COO), 1.99 (s, 3H, CH_3CONH) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.8, 170.7 (2C, CH_3CONH and CH_3COO), 169.7, 161.8 (C-1), 158.0-157.1 (q, J_{C-F} = 38 Hz, $COCF_3$), 144.7 (C-2), 119.0-112.2 (1C, CF_3), 108.5 (C-3), 73.6 (C-6), 71.4 (C-8), 68.1 (C-7), 62.0 (C-9), 52.5 ($COOCH_3$), 46.7 (C-5), 42.4 (C-4), 22.8 (CH_3CONH), 20.8, 20.7, 20.4 (3C, CH_3COO) ppm. MS (ESI positive) *m/z* 527.5 $[M+H]^+$, 549.4 $[M+Na]^+$, 1076.0 $[2M+Na]^+$. $C_{20}H_{25}F_3N_2O_{11}$: calcd. C, 45.63; H, 4.79; N, 5.32; found C, 45.54; H, 4.75; N, 5.16.

Starting from compound **14b** (105 mg, 0.2 mmol) and performing the reaction at 50 °C for 30 min in MeCN (Entry 9) the glycal **20** (78 mg, 78%) is obtained, as only stereoisomer and with all physico-chemical properties practically superimposable to those previously reported.

Performing the reaction at 50 °C for 30 min (Entry 3), the glycal **20** (83 mg, 83%) is also obtained, as only stereoisomer and with all physico-chemical properties practically superimposable to those previously reported.

Under TMSOTf catalysis*:* A solution of the glycal **19** (105 mg, 0.2 mmol) in MeCN (1.5 mL), containing TMSOTf (370 µL, 2.0 mmol), is heated at 50 °C for 180 min (Entry 20). Work-up and rapid chromatography, eluting with AcOEt/hexane (80:20 v/v), affords in the sequence the less polar glycal **21** (28 mg, 27%) followed by the more polar glycal **20** (58 mg, 55%), with all physico-chemical properties superimposable to those previously reported.

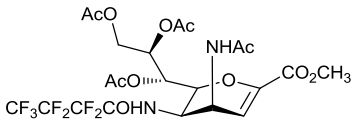
Preparation of methyl 4-acetamido-2,6-anhydro-5-(2,2,3,3,3-pentafluoropropanamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (25)



25

Starting from the glycal **23** (115 mg, 0.2 mmol) and operating at 50 °C for 30 min in MeCN (Entry 5), the title compound **25** is obtained, after rapid chromatography, eluting with AcOEt/hexane (70:30 v/v), as a white solid (98 mg, 85%): m.p. 144-147°C (from CH_2Cl_2-disopropylether); $[\alpha]_D^{20}$ – 39.2 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$) δ = 7.78 (1H, d, $J_{NH,5}$ = 9.0 Hz, $NHCOCF_2CF_3$), 6.47 (1H, d, $J_{NH,4}$ = 8.8 Hz, $NHCOCH_3$), 6.01 (1H, d, $J_{3,4}$ = 5.2 Hz, 3-H), 5.43 (1H, dd, $J_{7,6}$ = $J_{7,8}$ = 3.0 Hz, 7-H), 5.32 (1H, ddd, $J_{8\text{-}9a}$ = $J_{8,7}$ = 3.0, $J_{8,9b}$ = 8.2 Hz, 8-H), 4.90 (1H, dd, $J_{4,5}$ = $J_{4,3}$ = 5.2, $J_{4,NH}$ = 8.8 Hz, 4-H), 4.80 (1H , dd, $J_{9a,8}$ = 3.0, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.34 (1H, ddd, $J_{5,4}$ = 5.2, $J_{5,6}$ = $J_{5,NH}$ = 9.0 Hz, 5-H), 4.35 (1H, dd, $J_{6,7}$ = 3.0, $J_{6,5}$ = 9.0 Hz, 6-H), 4.17 (1H, dd, $J_{9b,8}$ = 8.2, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.77 (3H, s, $COOCH_3$), 2.08 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.97 (3H, s, CH_3CONH) ppm. ^{13}C NMR ($CDCl_3$) δ = 171.1($NHCOCH_3$), 170.8, 170.5 169.7 (3C, CH_3COO), 161.7 (C-1), 158.0 (t, $J_{C\text{-}F}$ = 26 Hz, $\underline{C}OCF_2$), 144.6 (C-2), 119.2-104.0 (2C, CF_2CF_3), 108.5 (C-3), 73.2 (C-6), 71.9 (C-8), 67.9 (C-7), 62.0 (C-9), 52.5 ($COOCH_3$), 46.7 (C-5), 42.4 (C-4), 22.7 (CH_3CONH), 20.8, 20.7, 20.3 (3C, CH_3COO) ppm. MS (ESI positive) *m/z* 577.2 $[M+H]^+$, 599.3 $[M+Na]^+$, 1175.7 $[2M+Na]^+$; (ESI negative) *m/z* 575.1 $[M-H]^-$. $C_{21}H_{25}F_5N_2O_{11}$: calcd C, 43.76; H, 4.37; N, 4.86; found C, 43.55; H, 4.26; N, 4.71.

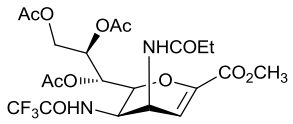
Preparation of methyl 4-acetamido-2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (26)



26

Starting from the glycal **24** (125 mg, 0.2 mmol) and operating at 50 °C for 30 min in MeCN (Entry 6), the title compound **26** is obtained, after rapid chromatography, eluting with AcOEt/hexane (70:30 v/v), as a white solid (101 mg, 81%): m.p. 158-160°C (from CH_2Cl_2-disopropylether); $[\alpha]_D$– 22.0 (*c* 1 in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ = 7.48 (1H, d, $J_{NH,5}$ = 8.9 Hz, $NHCOCF_2CF_2CF_3$), 6.22 (1H, d, $J_{NH,4}$ = 8.9 Hz, $NHCOCH_3$), 6.00 (1H, d, $J_{3,4}$ = 5.1 Hz, 3-H), 5.43 (1H, dd, $J_{7,6}$ = $J_{7,8}$ = 3.0 Hz, 7-H), 5.25 (1H, ddd, $J_{8\text{-}9a}$ = $J_{8,7}$ = 3.0, $J_{8,9b}$ = 7.8 Hz, 8-H), 4.89 (1H, dd, $J_{4,5}$ = $J_{4,3}$ = 5.1 Hz, $J_{4,NH}$ = 8.9 Hz, 4-H), 4.77 (1H , dd, $J_{9a,8}$ = 3.0, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.45 (1H, m, 5-H), 4.34 (1H, $J_{6,7}$ = 3.2, $J_{6,5}$ = 9.2 Hz, 6-H), 4.17 (1H, dd, $J_{9b,8}$ = 7.8, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.77 (3H, s, $COOCH_3$), 2.08 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 2.00 (3H, s, CH_3CONH) ppm. ^{13}C NMR ($CDCl_3$) δ = 171.1 ($NHCOCH_3$), 170.7, 170.5, 169.7 (3C, CH_3COO), 161.7 (C1), 157.9 (t, $J_{C\text{-}F}$ = 26 Hz, $COCF_2$), 144.6 (C-2), 119.2-116.0 (3C, $CF_2CF_2CF_3$), 108.7 (C-3), 73.5 (C-6), 71.9 (C-8), 68.1 (C-7), 62.0 (C-9), 52.5 ($COO\underline{C}H_3$), 46.9 (C-5), 42.6 (C-4), 22.7 (CH_3CONH), 20.8, 20.6, 20.3 (3C, $\underline{C}H_3COO$) ppm. MS (ESI positive) *m/z* 627.5 $[M+H]^+$, 649.6 $[M+Na]^+$, 1275.8 $[2M+Na]^+$; MS (ESI negative) *m/z* 625.3 $[M-H]^-$. $C_{22}H_{25}F_7N_2O_{11}$: calcd C, 42.18; H, 4.02; N, 4.47; found C, 42.30; H, 4.15; N, 4.71.

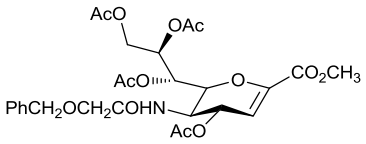
Preparation of methyl 2,6-anhydro-4-propoxycarbonilamido-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (22):



22

Starting from the glycal **19** (105 mg, 0.2 mmol) and operating at 50 °C for 30 min in EtCN (Entry 4), the title compound **22** is obtained, after rapid chromatography, eluting with AcOEt/hexane (70:30 v/v), as a white solid (80 mg, 78%): m.p. 129-131°C (from CH_2Cl_2-diisopropylether); $[\alpha]_D^{20}$ - 32.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.50 (d, $J_{NH,5}$ = 9.2 Hz, 1H, CF_3CONH), 6.30 (d, $J_{NH,4}$ = 8.5 Hz, 1H, CH_3CONH), 5.98 (d, $J_{3,4}$ = 5.2 Hz, 1H, 3-H), 5.48 (t, $J_{7,6}$ = $J_{7,8}$ = 3.4 Hz, 1H, 7-H), 5.26 (ddd, $J_{8,9a}$ = $J_{8,7}$ = 3.4, $J_{8,9b}$ = 8.1 Hz, 1H, 8-H), 4.91 (ddd, $J_{4,5}$ = $J_{4,3}$ = 5.2, $J_{4,NH}$ = 8.6 Hz, 1H, 4-H), 4.84 (dd, 1H, $J_{9a,8}$ = 3.4, $J_{9a,9b}$ = 12.3 Hz, 9a-H), 4.40 (ddd, $J_{5,4}$ = 5.2, $J_{5,6}$ = $J_{5,NH}$ = 9.2 Hz, 1H, 5-H), 4.32 (dd, $J_{6,7}$ = 3.4, $J_{6,5}$ = 9.2 Hz, 1H, 6-H), 4.13 (dd, $J_{9b,8}$ = 8.1, $J_{9b,9a}$ = 12.3 Hz, 1H, 9b-H), 3.79 (s, 3H, $COOCH_3$), 2.26-2.10 (m, 2H, CH_3CH_2CONH), 2.1 (s, 3H, CH_3COO), 2.04 (overlapping, 6H, 2XCH_3COO), 1.12 (t, $J_{CH3,CH2}$ = 7.6 Hz, 3H, $\underline{CH_3}CH_2CONH$) ppm. ^{13}C NMR ($CDCl_3$): δ = 174. 7 (CH_3CH_2NHCO), 171.4, 170.8, 169.6 (3XCH_3COO), 161.9 (C-1), 158.0-157.1 (q, J_{C-F} = 38 Hz, $\underline{C}OCF_3$), 144.6 (C-2), 119.0-112.0 (1C, CF_3), 108.5 (C-3), 73.6 (C-6), 71.1 (C-8), 68.0 (C-7), 61.8 (C-9), 52.6 ($COOCH_3$), 46.7 (C-5), 42.3 (C-4), 29.3 (CH_3CH_2CONH), 20.7 (2C, CH_3COO), 20.4 (CH_3COO), 9.6 (CH_3CH_2CONH) ppm. MS (ESI positive) *m/z* 541.0 $[M+H]^+$, 563.1 $[M+Na]^+$, 1103.6 $[2M+Na]^+$. $C_{21}H_{27}F_3N_2O_{11}$: calcd C, 46.67; H, 5.04; N, 5.18; found C, 46.37; H, 4.97; N, 5.08.

Preparation of methyl 2,6-anhydro-5-(*N*-benzyloxyacetyl)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (27)

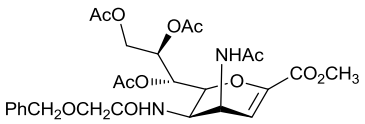


27

The glycal **19** (300 mg, 0.57 mmol) is treated with a saturated moist methanolic solution of K_2CO_3 (3.0 mL), at 23 °C for 24 h. Then, $BnOCH_2COCl$ (387 µL, 2.5 mmol) is added to the reaction mixture that is stirred at 23°C for 3 h. At this time, the reaction mixture is acidified by addition of aqueous HCl (0.1 M) and the solvent is removed under reduced pressure. The residue is dissolved in methanol (5.0 mL), treated with acidic resin (100 mg) (Dowex 50WX8, H^+) and stirred for 24 h, at 23 °C. Then, the resin was filtered, washed with methanol-water and the solvent was then removed under reduced pressure to afford a residue that was dissolved in pyridine (1.5 mL) and treated with Ac_2O (540 µL,5.7 mmol) for 12 h, at 23 °C. At this time the reaction mixture was poured into an ice cold aqueous NaCl solution and extracted with CH_2Cl_2, to afford, after a sequential washing, drying and solvent evaporation, a crude residue that by rapid chromatography (eluting with 1:1 v/v; AcOEt/hexane) gave the title compound 6d, as a white solid (118 mg, 55%): m.p. 124-126°C (from diisopropil ether) ; $[\alpha]_D$ + 25.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$) δ = 7.40-7.30 (5H, overlapping, OCH_2*Ph*), 6.66 (1H, d, $J_{NH,5}$ = 9.3 Hz, $NHCOCH_2$), 6.00 (1H, d, $J_{3,4}$ = 3.1 Hz, 3-H), 5.54-5.47 (2H, overlapping, 4-H and 7-H), 5.37 (1H, ddd, $J_{8\text{-}9a}$ = 3.2, $J_{8,7}$ = 5.3, $J_{8,9b}$ = 6.7 Hz, 8-H), 4.36-4.56 (3H, overlapping, 9a-H and OCH_2Ph), 4.47-4.38 (2H, overlapping, 5-H and 6-H), 4.18 (1H, dd, $J_{9b,8}$ = 6.7, $J_{9b,9a}$ = 12.3 Hz, 9b-H), 3.91 (2H, overlapping, $\underline{CH_2}CONH$), 3.80 (3H, s, $COOCH_3$), 2.21 (3H, s, CH_3COO), 2.07-2.04 (6H, overlapping, 2 X CH_3COO), 2.03 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) δ = 170.5, 170.0 (3C, CH_3COO), 169.9 ($NHCOCH_2$), 169.7 (CH_3COO), 161.5 (C-1), 145.1 (C-2), 136.7-128.0 (6C,

OCH_2*Ph*), 107.9 (C-3), 76.4 (C-6), 73.6 (OCH_2Ph), 70.3 (C-8), 69.2 ($NHCOCH_2$), 67.9 (C-7), 67.5 (C-4), 61.9 (C-9), 52.5 ($COOCH_3$), 46.0 (C-5), 20.8, 20.7 (4C, CH_3COO) ppm. MS (ESI positive) *m/z* 580.6 $[M+H]^+$, 602.6 $[M+Na]^+$. $C_{27}H_{33}NO_{13}$: calcd C, 55.96; H, 5.74; N, 2.42; found C, 56.10; H, 5.91; N, 2.52.

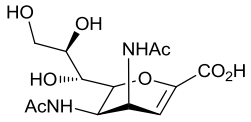
Preparation of methyl 4-acetamido-2,6-anhydro-5-(*N*-benzyloxyacetyl)-7,8,9-tri-*O*-acetyl-4,3,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (28)



28

Starting from the glycal 6d (116 mg, 0.2 mmol) and operating at 50 °C for 30 min in MeCN,the title compound 12 was obtained, after rapid chromatography, eluting with AcOEt/hexane (90:10 v/v), as a white solid (88 mg, 76%): m.p. 131-122°C; $[\alpha]_D^{20}$ - 33.1 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ = 7.40-7.30 (5H, overlapping, OCH_2*Ph*), 6.66 (1H, d, $J_{NH,5}$ = 10.0 Hz, $NHCOCH_2$), 6.01 (1H, d, $J_{3,4}$ = 5.2 Hz, 3-H), 5.50-5.43 (2H, overlapping, $NHCOCH_3$ and 7-H), 5.32-5.28 (1H, m, 8-H), 4.88-4.82 (1H, m, 4-H), 4.68 (1H, dd, $J_{9a,8}$ = 2.5, $J_{9a,9b}$ = 12.3 Hz, 9a-H), 4.59-4.52 (2H, overlapping, OCH_2Ph), 4.48 (1H, ddd, $J_{5,4}$ = 5.0, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.17 (1H, dd, $J_{9b,8}$ = 7.1, $J_{9b,9a}$ = 12.3 Hz, 9b-H), 4.09 (1H, dd, $J_{6,7}$ = 2.9, $J_{6,5}$ = 10.0 Hz, 6-H), 3.94 (2H, overlapping, CH_2CONH), 3.80 (3H, s, $COOCH_3$), 2.11 (3H, s, CH_3COO), 2.08 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 1.91 (3H, s, CH_3CONH) ppm. ^{13}C NMR ($CDCl_3$) δ = 170.6, 170.3 170.1 (3C, CH_3COO), 170.0 ($NHCOCH_3$), 169.9 ($NHCOCH_2$), 161.7 (C-1), 145.1 (C-2), 137.0-127.8 (6C, OCH_2*Ph*), 108.4 (C-3), 74.6 (C-6), 73.5 (OCH_2Ph), 71.3 (C-8), 69.5 ($NHCOCH_2$), 68.0 (C-7), 62.1 (C-9), 52.5 ($COOCH_3$), 44.8 (C-5), 43.2 (C-4), 23.2 (CH_3CONH), 20.9, 20.7 (3C, CH_3COO) ppm. MS (ESI positive) *m/z* 579.6 $[M+H]^+$, 601.7 $[M+Na]^+$. $C_{27}H_{34}N_2O_{12}$: calcd C, 56.05; H, 5.92; N, 4.84; found C, 56.28; H, 6.01; N, 4.99.

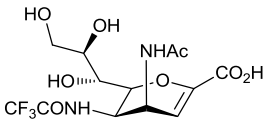
Preparation of 4,5-Diacetamido-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enoic acid (18a)



18a

The title compound **18a** is prepared by complete hydrolysis of glycal **20** (72 mg, 0.14 mmol) performed in a saturated moist methanolic solution of K_2CO_3 (0.5 mL), kept a 23 °C for 12 h. Then, the free amino acid formed, is amidated by addition of CH_3COCl (50 µL, 0.7 mmol) at 23°C for 30 min. The reaction mixture is treated with acidic resin (Dowex 50WX8, H^+) until acidic pH, then, the resin is filtered and washed with methanol. The solvent is removed under reduced pressure to afford glycal **11a**, as a white solid (37 mg, 82%): m.p. 105-107°C (from MeOH,-diisopropylether); $[\alpha]_D$ - 48.2 (*c* 1 in $CHCl_3$). ^1H NMR (D_2O): δ = 6.05, *J*3,4 = 5.6 Hz, 1H, 3-H), 4.77 (under water signal, 1H, 4-H), 4.36 (dd, $J_{5,4}$ = 4.7, $J_{5,6}$ = 10.7 Hz, 1H, 5-H), 4.19 (d app, $J_{6,5}$ = 10.7 Hz, 1H, 6-H), 3.96 (m, 1H, 8-H), 3.89 (dd, $J_{9a,8}$ = 1.9, $J_{9a,9b}$ = 11.8 Hz, 1H, 9a-H), 3.70 (d app, $J_{7,8}$ = 10.0 Hz, 1H, 7-H), 3.67 (dd, $J_{9b,8}$ = 6.1, $J_{9b,9a}$ = 11.8 Hz, 1H, 9b-H), 2.03 (s, 3H, CH_3CONH), 1.96 (s, 3H, CH_3CONH) ppm. ^{13}C NMR (D_2O): δ = 175.8 ($NHCOCH_3$), 174.5 ($NHCOCH_3$), 166.2 (C-1), 145.9 (C-2), 108.3 (C-3), 73.0 (C-6), 70.8 (C-8), 68.8 (C-7), 63.7 (C-9), 46.9 (C-5), 43.0 (C-4), 22.6 (CH_3CONH), 22.5 (CH_3CONH) ppm. MS (ESI negative) *m/z* 331.4 [M-H]-. $C_{13}H_{20}N_2O_8$: calcd C, 46.99; H, 6.07; N, 8.43; found C, 47.05; H, 5.96; N, 8.23.

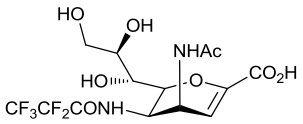
Preparation of 4-Acetamido-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enoic acid (18b):



18b

the title compound **18b** is prepared by selective hydrolysis of glycal **20** (72 mg, 0.14 mmol) performed in a solution of methanol-water (1.5 mL, 2:1 v/v) containing Et_3N (0.90 mL), kept a 23°C for 12 h. Then the solvent is removed under reduced pressure and the residue recovered with aqueous methanol and lyophilized, to afford glycal **18b** as a white solid (48 mg, 90%): m.p. 129-131°C (from MeOH,-diisopropylether); $[\alpha]_D$ - 36.2 (*c* 1 in $CHCl_3$). ^1H NMR (D_2O): δ = 5.73(d, $J_{3,4}$ = 5.0 Hz, 1H, 3-H), 4.81 (under water signal, 1H, 4-H), 4.48 (dd, $J_{5,4}$ = 5.0, $J_{5,6}$ = 11.1 Hz, 1H, 5-H), 4.30 (d app, $J_{6,5}$ = 11.1 Hz, 1H, 6-H), 4.00 (ddd, $J_{8,9a}$ = 2.5, $J_{8,9b}$ = 6.4, *J*8,7 = 9.1 Hz, 1H, 8-H), 3.91 (d app, $J_{9a,9b}$ = 11.9 Hz, 1H, 9a-H), 3.65 (dd, $J_{9b,8}$ = 6.4, $J_{9b,9a}$ = 11.9 Hz, 1H, 9b-H), 3.60 (d app, $J_{7,8}$ = 9.1 Hz, 1H, 7-H), 1.94 (s, 3H, CH_3CONH) ppm. ^{13}C NMR (D_2O): δ = 174.5 ($NHCOCH_3$), 170.0 (C-1), 159.9 (t, J_{C-F} = 26 Hz, $COCF_2$), 150.3 (C-2), 120.0-105.0 (1C, CF_3), 103.1 (C-3), 71.4 (C-6), 70.7 (C-8), 69.1 (C-7), 63.7 (C-9), 48.4 (C-5), 42.9 (C-4), 22.4 (CH_3CONH) ppm.MS (ESI negative) *m/z* 385.1 [M-H]-. $C_{13}H_{17}F_3N_2O_8$: calcd C, 40.42; H, 4.44; N, 7.25; found C, 40.11; H, 4.60; N, 7.30.

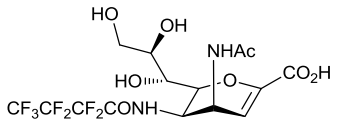
Preparation of 4-Acetamido-2,6-anhydro-5-(2,2,3,3,3-pentafluoropropanamido)-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enoic acid (18c):



18c

the title compound **18c** is prepared by selective hydrolysis of glycal **25** (79 mg, 0.14 mmol) performed in a solution of methanol-water (1.5 mL, 2:1 v/v) containing Et_3N (0.90 mL), kept a 23°C for 12 h. Then the solvent is removed under reduced pressure and the residue recovered with aqueous methanol and lyophilized, to afford glycal 18b as a white solid (48 mg, 90%): m.p. 126-128°C (from MeOH,-diisopropylether); $[\alpha]_D$ - 31.2 (*c* 1 in $CHCl_3$). ^1H NMR (D_2O) δ = 5.89 (1H, d, $J_{3,4}$ = 5.9 Hz, 3-H), 4.41-4.37 (2H, overlapping, 6-H and 5-H), 4.31 (1H, dd, $J_{4,5}$ = 1.1, $J_{4,3}$ = 5.8 Hz, 4-H), 4.00 (1H, ddd, $J_{8\text{-}9a}$ = 2.5, $J_{8,9b}$ = 6.5, $J_{8,7}$ = 9.2 Hz, 8-H), 3.90 (1H, dd, $J_{9a,8}$ = 2.5, $J_{9a,9b}$ = 11.9 Hz, 9a-H), 3.65 (1H, dd, $J_{9b,8}$ = 6.5, $J_{9b,9a}$ = 11.9 Hz, 9b-H), 3.57 (1H, d app, $J_{7,8}$ = 9.5 Hz, 7-H) ppm. ^{13}C NMR (MeOD) δ = 170.1 (C-1), 159.7 (t, $J_{C\text{-}F}$ = 26 Hz, $\underline{C}OCF_2$), 149.8 (C-2), 120.0-108.2 (2C, CF_2CF_3), 104.1 (C-3), 70.9 (C-6), 70.6 (C-8), 69.0 (C-7), 63.7 (C-9), 61.1 (C-5), 49.4 (C-4) ppm. MS (ESI negative) *m/z* 394.2 $[M\text{-}H]^-$. $C_{12}H_{14}F_5NO_8$: calcd C, 36.47; H, 3.57; N, 3.54; found C, 36.09; H, 3.69; N, 3.66.

Preparation of 4-Acetamido-2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enoic acid (18d):



18d

the title compound **18d** is prepared by selective hydrolysis of glycal **26** (83 mg, 0.14 mmol) performed in a solution of methanol-water (1.5 mL, 2:1 v/v) containing Et_3N (0.90 mL), kept a 23°C for 12 h. Then the solvent is removed under reduced pressure and the residue recovered with aqueous methanol and lyophilized, to afford glycal **18d** as a white solid m.p. 116-119°C; $[\alpha]_D^{20}$ – 28.6 (*c* 1 in MeOH); ^{1}H NMR (D_2O) δ = 5.84 (1H, d, $J_{3,4}$ = 5.7 Hz, 3-H), 4.43 (1H, d app, $J_{6,5}$ = 11.5 Hz, 6-H), 4.29 (1H, dd, $J_{5,4}$ = 3.8, $J_{5,6}$ = 11.5 Hz, 5-H), 4.18 (1H, dd, $J_{4,5}$ = 3.8, $J_{4,3}$ = 5.7 Hz, 4-H), 3.90 (1H, ddd, $J_{8,9a}$ = 2.7, $J_{8,9b}$ = 5.7, $J_{8,7}$ = 8.9 Hz, 8-H), 3.85 (1H, dd, $J_{9a\text{-}8}$ = 2.7, $J_{9a,9b}$ = 11.4 Hz, 9a-H), 3.63 (1H, dd, $J_{9b,8}$ = 5.7, $J_{9b,9a}$ = 11.4 Hz, 9b-H), 3.50 (1H, d app, $J_{7,8}$ = 8.9 Hz, 7-H) ppm. ^{13}C NMR (D_2O) δ = 170.3 (C-1), 159.1 (t, $J_{C\text{-}F}$ = 26 Hz, $\underline{C}OCF_2$), 151.3 (C-2), 124.0-108.2 (3C, CF_2CF2CF_3), 104.9 (C-3), 71.9 (C-6), 71.7 (C-8), 70.3 (C-7), 65.1 (C-9), 61.9 (C-5), 50.4 (C-4) ppm. MS (ESI negative) *m/z* 444.3 $[M\text{-}H]^-$. $C_{13}H_{14}F_7NO_8$: calc C, 35.07; H, 3.17; N, 3.15; found C, 35.29; H, 3.33; N, 3.31.

Theorical Calculations

A search of the conformational space of the intermediate allylic carbocation is performed using quantum mechanical AM1 method implemented within the HyperChem software package, varying all the dihedral angles in a Metropolis Monte-Carlo conformational search. The search is performed with a range for acyclic or ring torsion variation of ±10–120°. The Random Walk, and Metropolis Criterion use T = 300 K, switching to 400 K. The 10 lowest energy conformations found for each $^5H_6$ and $^6H_5$ C (with 5,6 pseudodiequatorial

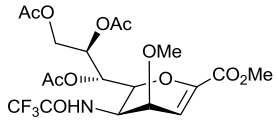
chains conformers are fully re-optimized in the Gaussian 03 program package by a DFT approach at the B3LYP level. The 6-31G(d) basis set is used for all the atom of molecules. Vibrational frequencies are computed, for all conformers, at the same level of theory to verify that the optimized structures are minima of potential energy.

5.3.4 General access to C-4 position of 2,3 unsaturated *N*-Acetylneuraminic acid *via* direct nucleophilic substitution

General procedure

The the *N*-trifluoroacetyl glycal **19** (0.20 mmol), dissolved in DCM (0.60 mL), is reacted with the appropriate nucleophile (2.0 mmol) and BF_3Et_2O (246 µL, 2.0 mmol) at 40 °C. The reaction mixture is cooled, and evaporated under reduced pressure. The obtained crude residue is purified by flash chromatography, to afford the appropriate glycal.

Preparation of methyl 4-methoxy-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (29)

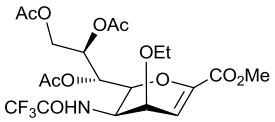


29

Starting from glycal **19** (105 mg, 0.2 mmol) and MeOH (81 µL, 2.0 mmol), after 15 min heating at 40 °C in DCM the glycal **29** is obtained as a white solid (85.8 mg, 81%). Glycal **29** shows: m.p. 122-124°C; $[\alpha]_D$ - 11.3 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 6.71 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.26 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.39 (1H, dd, $J_{7,6}$ = 2.1, $J_{7,8}$ = 5.0 Hz, 7-H), 5.33 (1H, ddd, $J_{8,9a}$ = 2.8, $J_{8,7}$ = 5.0, $J_{8\text{-}9b}$ = 6.9 Hz, 8-H), 4.66 (1H, dd, $J_{9a,8}$ = 2.8, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.39 (1H, ddd, $J_{5,4}$ = 4.0, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.33 (1H, dd, $J_{6,7}$ = 2.1, $J_{6,5}$ = 10.0 Hz, 6-H), 4.14

(1H, dd, $J_{9b,8}$ = 6.9, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.82 (3H, s, $COOCH_3$), 3.80 (1H, dd, , $J_{4,5}$ = 4.0, $J_{4,3}$ = 5.3 Hz, 4-H), 3.33 (3H, s, OCH_3), 2.10 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.2, 169.8 (3C, $COCH_3$), 161.8 (C-1), 157.4 (q, J_{C-F} = 38 Hz, $COCF_3$), 145.9 (C-2), 119.0-110.0 (CF_3), 105.9 (C-3), 73.1 (C-6), 71.0 (C-8), 69.2 (C-4), 67.6 (C-7), 62.1 (C-9), 56.6 (OCH_3), 52.7 ($COOCH_3$), 46.3 (C-5), 20.9, 20.7, 20.6 (3C, CH_3COO) ppm. MS (ESI positive) *m/z* 500.6 $[M+H]^+$, 523.5 $[M+Na]^+$. $C_{19}H_{24}F_3NO_{11}$: calcd C, 45.70; H, 4.84; N, 2.80; found C, 45.88; H, 5.02 N, 2.88.

Preparation of methyl 4-ethyl-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (30)

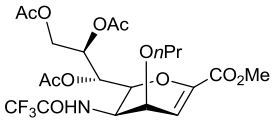


30

Starting from glycal **19** (105 mg, 0.2 mmol) and EtOH (117 µL, 2.0 mmol), after 15 min heating at 40 °C in DCM the glycal **30** is obtained as a white solid (81.1 mg, 79%). Glycal **30** shows: m.p. 135-137°C; $[\alpha]_D$ - 55.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.71 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.24 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.41 (1H, dd, $J_{7,6}$ = 2.1, $J_{7,8}$ = 4.9 Hz, 7-H), 5.33 (1H, ddd, $J_{8,9a}$ = 2.8, $J_{8,7}$ = 4.9, $J_{8\text{-}9b}$ = 7.1 Hz, 8-H), 4.69 (1H, dd, $J_{9a,8}$ = 2.8, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.37 (1H, ddd, $J_{5,4}$ = 3.9, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.23 (1H, dd, $J_{6,7}$ = 2.1, $J_{6,5}$ = 10.0 Hz, 6-H), 4.15 (1H, dd, $J_{9b,8}$ = 7.1, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.89 (1H, dd, $J_{4,5}$ = 3.9, $J_{4,3}$ = 5.3 Hz, 4-H), 3.82 (3H, s, $COOCH_3$), 3.76 (1H, m, OCH_2CH_3), 3.50 (1H, m, OCH_2CH_3), 2.11 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 1.22 (3H, m, OCH_2CH_3) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.2, 169.7 (3C, $COCH_3$), 161.8 (C-1), 157.4 (q, J_{C-F} = 38 Hz, $COCF_3$), 145.6 (C-2), 119.0-110.0 (CF_3), 106.8 (C-3), 73.1 (C-6), 71.1 (C-8), 67.7 (C-4), 67.6 (C-7), 64.8 (OCH_2CH_3), 62.1 (C-9), 52.6 ($COOCH_3$), 46.2 (C-5), 20.9, 20.7, 20.6 (3C, CH_3COO), 15.3 (OCH_2CH_3) ppm.

MS (ESI positive) *m/z* 514.5 $[M+H]^+$, 536.4 $[M+Na]^+$. $C_{20}H_{26}F_3NO_{11}$: calcd C, 46.79; H, 5.10; N, 2.73; found C, 46.51; H, 5.01 N, 2.50.

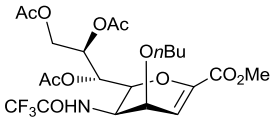
Preparation of methyl 4-*n*-propyl-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,5-diideoxy-D-*glycero*-D-*talo*-non-2-enonate (31)



31

Starting from glycal **19** (105 mg, 0.2 mmol) and *n*PrOH (150 µL, 2.0 mmol), after 30 min heating at 40 °C in DCM the glycal **31** is obtained as a white solid (74.9 mg, 71%). Glycal **31** shows: m.p. 124-127°C; $[\alpha]_D^{20}$ – 38.2 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.71 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.24 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.41 (1H, dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 5.0 Hz, 7-H), 5.35 (1H, ddd, $J_{8,9a}$ = 2.8, $J_{8,7}$ = 5.0, $J_{8\text{-}9b}$ = 6.9 Hz, 8-H), 4.68 (1H, dd, $J_{9a,8}$ = 2.8, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.38 (1H, ddd, $J_{5,4}$ = 3.9, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.32 (1H, dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.0 Hz, 6-H), 4.15 (1H, dd, $J_{9b,8}$ = 6.9, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.88 (1H, dd, $J_{4,5}$ = 3.9, $J_{4,3}$ = 5.3 Hz, 4-H), 3.81 (3H, s, $COOCH_3$), 3.67 (1H, m, $OCH_aCH_2CH_3$), 3.38 (1H, m, $OCH_bCH_2CH_3$), 2.10 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 1.60 (2H, m, $OCH_2CH_2CH_3$), 0.92 (3H, m, $OCH_2CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.1, 169.7 (3C, $COCH_3$), 161.8 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 145.5 (C-2), 119.0-110.0 (CF_3), 106.8 (C-3), 73.1 (C-6), 71.0 (C-8), 70.9 (OCH_2CH_2), 67.8 (C-7), 67.6 (C-4), 62.1 (C-9), 52.6 ($COOCH_3$), 46.3 (C-5), 22.9 ($OCH_2CH_2CH_3$), 20.9, 20.7, 20.6 (3C, CH_3COO), 10.4 ($OCH_2CH_2CH_3$) ppm. MS (ESI positive) *m/z* 528.4 $[M+H]^+$, 551.4 $[M+Na]^+$. $C_{21}H_{28}F_3NO_{11}$: calcd C, 47.82; H, 5.35; N, 2.66; found C, 47.69; H, 5.43 N, 2.62.

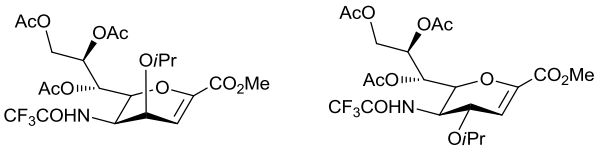
Preparation of methyl 4-*n*-butyl-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (32)



32

Starting from glycal **19** (105 mg, 0.2 mmol) and *n*BuOH (183 µL, 2.0 mmol), after 30 min heating at 40 °C in DCM the glycal **32** is obtained as a white solid (80.1 mg, 74%). Glycal **32** shows: m.p. 118-121°C; $[\alpha]_D$ – 36.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.72 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.24 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.41 (1H, dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 4.9 Hz, 7-H), 5.34 (1H, ddd, $J_{8,9a}$ = 2.8, $J_{8,7}$ = 4.9, $J_{8\text{-}9b}$ = 7.0 Hz, 8-H), 4.67 (1H, dd, $J_{9a,8}$ = 2.8, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.37 (1H, ddd, $J_{5,4}$ = 4.0, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.32 (1H, dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.0 Hz, 6-H), 4.16 (1H, dd, $J_{9b,8}$ = 7.0, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.87 (1H, dd, $J_{4,5}$ = 4.0, $J_{4,3}$ = 5.3 Hz, 4-H), 3.82 (3H, s, $COOCH_3$), 3.70 (1H, m, $OC\underline{H_a}(CH_2)_2H_3$), 3.42 (1H, m, $OC\underline{H_b}(CH_2)_2CH_3$), 2.11 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 1.60-1.52 (2H, m, $OCH_2C\underline{H_2}CH_2CH_3$), 1.42-1.31 (2H, m, $O(CH_2)_2C\underline{H_2}CH_3$), 0.93 (3H, m, $OCH_2CH_2C\underline{H_3}$) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.2, 169.7 (3C, $COCH_3$), 161.8 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 145.5 (C-2), 119.0-110.0 (CF_3), 106.8 (C-3), 73.2 (C-6), 71.1 (C-8), 69.1 ($O\underline{C}H_2(CH_2)_2CH_£$), 67.8 (C-7), 67.6 (C-4), 62.1 (C-9), 52.6 ($COOCH_3$), 46.3 (C-5), 31.7 ($OCH_2\underline{C}H_2CH_2CH_3$), 20.9, 20.7, 20.6 (3C, $\underline{CH_3}COO$), 19.2 ($O(CH_2)_2\underline{C}H_2CH_3$), 13.7 ($O(CH_2)_3CH_3$) ppm. MS (ESI positive) *m/z* 576.4 $[M+H]^+$, 598.5 $[M+Na]^+$. $C_{25}H_{28}F_3NO_{11}$: calcd C, 52.18; H, 4.90; N, 2.43; found C, 52.07; H, 5.00; N, 2.32.

Preparation of methyl 4-*i*-propyl-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (33, 4-β) and methyl 4- *i*-propyl-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (33, 4-α)

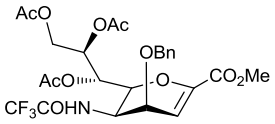


33 (4-β) 33 (4-α)

Starting from glycal **19** (105 mg, 0.2 mmol) and *i*PrOH (153 µL, 2.0 mmol), after 60 min heating at 40 °C in DCM the), a mixture of the glycals **33 (4-β)** and **33 (4-α)** is obtained that, after rapid chromatography, eluting with hexane/AcOEt (80:20 v/v), afforded in the sequence, the less polar glycal **33 (4-β)** (52.7 mg, 50%) followed by the more polar glycal **33 (4-α)** (26.4 mg, 25%), both in pure form. Glycal **33 (4-β)** shows: m.p. 141-143°C; $[\alpha]_D$ - 24.2 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.70 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.17 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.41 (1H, dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 4.8 Hz, 7-H), 5.34 (1H, ddd, $J_{8,9a}$ = 2.8, $J_{8,7}$ = 4.8, $J_{8\text{-}9b}$ = 7.1 Hz, 8-H), 4.70 (1H, dd, $J_{9a,8}$ = 2.8, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.34 (1H, ddd, $J_{5,4}$ = 4.0, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.29 (1H, dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.0 Hz, 6-H), 4.16 (1H, dd, $J_{9b,8}$ = 7.1, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.95 (1H, dd, , $J_{4,5}$ = 4.0, $J_{4,3}$ = 5.3 Hz, 4-H), 3.84-3.75 (4H, overlapping, $COOCH_3$ and $OC\underline{H}(CH_3)_2$), 2.10 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 1.20 (3H, d, $J_{CH,(CH3)a}$ = 6.1 Hz, $OCH(C\underline{H}_3)a$), 1.16 (3H, d, $J_{CH,(CH3)b}$= 6.1 Hz, $OCH(C\underline{H}_3)b$) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.2, 169.7 (3C, $\underline{C}OCH_3$), 161.9 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 145.3 (C-2), 119.0-105.5 (CF_3), 107.7 (C-3), 73.1 (C-6), 71.4 ($O\underline{C}H(CH_3)_2$), 71.1 (C-8), 67.7 (C-7), 65.8 (C-4), 62.1 (C-9), 52.6 ($COOCH_3$), 46.2 (C-5), 23.3 ($OCH(\underline{C}H_3)a$), 21.7 ($OCH(\underline{C}H_3)b$), 20.9, 20.7, 20.6 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 528.3 $[M+H]^+$, 551.4 $[M+Na]^+$. $C_{21}H_{28}F_3NO_{11}$ calcd C, 47.82; H, 5.35; N, 2.66; found C, 47.62; H, 5.48 N, 2.49. Glycal **33 (4-α)** shows: m.p. 136-138°C; $[\alpha]_D$ + 33.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.65 (1H, d,

$J_{NH,5}$ = 7.5 Hz, N-H), 6.10 (1H, d, $J_{3,4}$ = 3.3 Hz, 3-H), 5.48 (1H, dd, $J_{7,6}$ = $J_{7,8}$ = 5.5 Hz, 7-H), 5.39 (1H, ddd, $J_{8,9a}$ = 3.9, $J_{8,7}$ = $J_{8\text{-}9b}$ = 5.5 Hz, 8-H), 4.56 (1H, dd, $J_{6,7}$ = 5.5, $J_{6,5}$ = 7.5 Hz, 6-H), 4.49 (1H, dd, $J_{9a,8}$ = 3.9, $J_{9a,9b}$ = 12.2 Hz, 9a-H), 4.33 (1H, dd,, $J_{4,3}$ = 3.3, $J_{4,5}$ = 7.5, Hz, 4-H), 4.20 (1H, dd, $J_{9b,8}$ = 5.5, $J_{9b,9a}$ = 12.2 Hz, 9b-H), 3.96 (1H, ddd, $J_{5,4}$ = $J_{5,6}$ = $J_{5,NH}$ = 7.5 Hz, 5-H), 3.84-3.77 (4H, overlapping, $COOCH_3$ and $OC\underline{H}(CH_3)_2$), 2.12 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.18 (3H, d, $J_{CH,(CH3)a}$ = 6.1 Hz, $OCH(C\underline{H}_3)a$), 1.15 (3H, d, $J_{CH,(CH3)b}$= 6.1 Hz, $OCH(C\underline{H}_3)b$) ppm. ^{13}C NMR ($CDCl_3$) 170.5, 170.2, 169.7 (3C, $\underline{C}OCH_3$), 161.8 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 143.2 (C-2), 119.0-105.5 (CF_3), 109.8 (C-3), 74.5 (C-6), 71.7 ($O\underline{C}H(CH_3)_2$), 69.7 (C-8), 68.8 (C-4), 67.9 (C-7), 65.8 (C-4), 61.6 (C-9), 52.5 ($COOCH_3$), 50.1 (C-5), 22.9 ($OCH(\underline{C}H_3)a$), 22.7 ($OCH(\underline{C}H_3)b$), 20.8, 20.7, 20.5 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 528.3 $[M+H]^+$, 551.4 $[M+Na]^+$. $C_{21}H_{28}F_3NO_{11}$ calcd C, 47.82; H, 5.35; N, 2.66; found C, 47.62; H, 5.48 N, 2.49.

Preparation of methyl 4-benzyl-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (34)

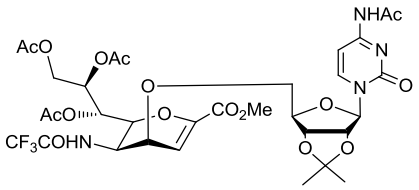


34

Starting from glycal **19** (105 mg, 0.2 mmol) and BnOH (207 µL, 2.0 mmol), after 30 min heating at 40 °C in DCM the glycal **34** is obtained as a white solid (95.5 mg, 83%). Glycal **34** shows: m.p. 119-121°C; $[\alpha]_D$ – 31.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.41-7.24 (5H, overlapping, $CH_2\underline{Ph}$), 6.72 (1H, d, $J_{NH,5}$ = 8.0 Hz, N-H), 6.26 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.39 (1H, dd, $J_{7,6}$ = 1.4, $J_{7,8}$ = 5.2 Hz, 7-H), 5.34 (1H, ddd, $J_{8,9a}$ = 2.8, $J_{8,7}$ = 5.2, $J_{8\text{-}9b}$ = 6.8 Hz, 8-H), 6.73 (1H, d, $J_{CHa\text{-}CHb}$ = 11.4 Hz, $OC\underline{H}_aPh$), 4.67 (1H, dd, $J_{9a,8}$ = 2.8, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.49 (1H, d, $J_{CHb\text{-}CHb}$ = 11.4 Hz, $OC\underline{H}_bPh$), 4.41-4.33 (2H, overlapping, 5-H and 6-H), 4.14 (1H, dd, $J_{9b,8}$

= 6.8, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.97 (1H, m, 4-H), 3.83 (3H, s, $COOCH_3$), 2.09 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.1, 169.7 (3C, $COCH_3$), 161.8 (C-1), 157.4 (q, J_{C-F} = 38 Hz, $\underline{C}OCF_3$), 145.9 (C-2), 136.6-128.2 (6C, Ph), 119.0-110.0 (CF_3), 106.2 (C-3), 73.1 (C-6), 71.1 ($O\underline{C}H_2Ph$), 70.9 (C-8), 67.5 (7-C), 66.9 (C-4), 62.1 (C-9), 52.6 ($COOCH_3$), 46.2 (C-5), 20.9, 20.7, 20.6 (3C, $\underline{C}H_3COO$) ppm. MS (ESI positive) *m/z* 576.4 $[M+H]^+$, 598.5 $[M+Na]^+$. $C_{25}H_{28}F_3NO_{11}$: calcd C, 53.55; H, 5.66; N, 2.31; found C, 53.07; H, 5.13; N, 2.02.

Preparation of methyl 4-[6-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)]-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (35)

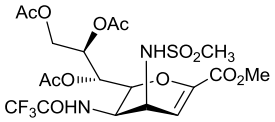


35

Starting from glycal **19** (105 mg, 0.2 mmol) and protected cytidine (125 mg, 0.4 mmol), after 120 min heating at 40 °C in DCM the glycal **35** is obtained as a white solid (91.9 mg, 58%). Glycal **34**: m.p. 129-131°C; $[\alpha]_D$ – 63.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 9.67 (1H, br s, N$\underline{H}$Ac), 9.29 (1H, br s, N$\underline{H}$$COCF_3$), 7.52 (1H, *J* = 7.38 Hz, H), 7.46 (1H, *J* = 7.38 Hz, H), 6.19 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.70-5.61 (2H, overlapping, 7-H and 8-H), 5.55 (1H, d, $J_{1'-2'}$ = 6.7 Hz, 1'-H), 5.17 (1H, m, 2'-H), 5.11 (1H, dd, $J_{9a,8}$ = 1.6, $J_{9a,9b}$ = 12.1 Hz, 9a-H), 4.43-4.31 (2H, overlapping, 5-H and 6-H), 4.19-4.09 (3H, overlapping, 3'-H, 4'-H and 9b-H), 4.01 (1H, dd, $J_{5'a,4'}$= 1.8, $J_{5'a,5'b}$= 10.3 Hz, 5'a-H), 3.88 (1H, dd, $J_{4,5}$ = 3.8, $J_{4,3}$ = 5.3 Hz, 4-H), 3.79 (3H, s, $COOCH_3$), 3.47 (1H, dd, $J_{5'b,4'}$= 1.1, $J_{5'b,5'a}$ = 10.3 Hz, 5'b-H), 2.22 (3H, s, CH_3COO), 2.13 (3H, s, CH_3COO), 2.11 (3H, s, CH_3COO), 2.0 (3H, s, CH_3CONH), 1.54 (3H, s, $CC\underline{H}_3$), 1.34 (3H, s, $CC\underline{H}_3$) ppm. ^{13}C NMR ($CDCl_3$) 170.4, 170.8, 170.5, 169.5 (5C, 3 X $COCH_3$, 2 X CONH), 163.9 (C-NHAc),

161.9 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 148.8 (N_2CO), 145.5 (C-2), 119.0-110.0 (CF_3), 114.2 115.3($\underline{C}(CH_3)_2$), 106.3 (C-3), 99.3 (CH), 97.1 (C-1'), 88.0, 83.8, 80.0 (C-3'), 72.8 (C-6), 72.5 (C-8), 69.0, 68.7, 67.1 (C-4), 62.1 (C-9), 52.5 ($COOCH_3$), 46.7 (C-5), 27.5 24.8 (2C, $C(\underline{C}H_3)_2$), 24.6 ($NHCOCH_3$), 21.3, 20.8, 20.6 (3C, $\underline{CH_3}COO$), ppm. MS (ESI positive) *m/z* 7.93.7 $[M+H]^+$, 816.6 $[M+Na]^+$. $C_{32}H_{39}F_3N_4O_{16}$: calcd C, 48.93; H, 5.45; N, 5.19; found C, 48.71; H, 5.20 N, 4.98.

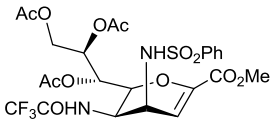
Preparation of methyl 4-methylsulfonamido-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (36)



36

Starting from glycal **19** (105 mg, 0.2 mmol) and $MeSO_2NH_2$ (190 mg, 2.0 mmol), after 15 min heating at 40 °C in DCM the glycal **36** is obtained as a white solid (97.7 mg, 87%). Glycal **36** shows: m.p. 155-157°C; $[\alpha]_D$ – 72.8 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 7.63 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.09 (1H, d, $J_{3,4}$ = 5.7 Hz, 3-H), 5.58 (1H, d, $J_{NH,4}$ = 9.0 Hz, H-NSO_2), 5.48 (1H, dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 4.1 Hz, 7-H), 5.36 (1H, ddd, $J_{8,9a}$ = 2.7, $J_{8,7}$ = 4.1, $J_{8,9b}$ = 6.9 Hz, 8-H), 4.78 (1H, dd, $J_{9a,8}$ = 2.7, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.40 (1H, ddd, $J_{5,4}$ = 4.7, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.28 (1H, dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.0 Hz, 6-H), 4.19-4.10 (2H, overlapping, 9b-H and 4-H), 3.82 (3H, s, $COOCH_3$), 3.03 (3H, s, $NHSO_2CH_3$), 2.09 (3H, s, CH_3COO), 2.08 (3H, s, $COOCH_3$), 2.05 (3H, s, $COOCH_3$) ppm. ^{13}C NMR ($CDCl_3$) 171.0, 170.9,169.8 (3C, 3 X $\underline{CO}CH_3$), 161.6 (C-1), 157.6 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_2$), 145.5 (C-2), 119.0-110.0 (CF_3), 106.7 (C-3), 72.9 (C-6), 71.4 (C-8), 67.9 (C-7), 62.2 (C-9), 52.8 ($COOCH_3$), 47.1 (C-4), 46.3 (C-5), 41.2 ($SO_2\underline{CH_3}$), 20.9, 20.7, 20.4 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 563.7 $[M+H]^+$, 585.5 $[M+Na]^+$, 1150.1 $[2M+Na]^+$. $C_{19}H_{25}F_3N_2O_{12}S$: calcd C, 40.57; H, 4.48; N, 4.98; found C, 40.88; H, 4.57; N, 5.11.

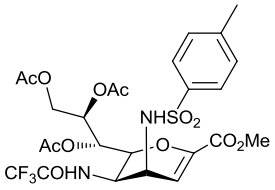
Preparation of methyl 4-benzylsulfonamido-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (37)



37

Starting from glycal **19** (105 mg, 0.2 mmol) and $PhSO_2NH_2$ (314 mg, 2.0 mmol), after 15 min heating at 40 °C in DCM the glycal **37** is obtained as a white solid (109.9 mg, 88%). Glycal **37** shows: m.p. 127-129°C; $[\alpha]_D^{20}$ – 15.7 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.88-7.83 (2H, overlapping, $SO_2\underline{Ph}$), 7.66 (1H, m, $SO_2\underline{Ph}$), 7.59-7.53 (2H, overlapping, $SO_2\underline{Ph}$), 7.40 (1H, d, $J_{NH,5}$ = 10.4 Hz, N-H), 5.52 (1H, d, $J_{3,4}$ = 5.8 Hz, 3-H), 5.50-5.43 (2H, overlapping, H-NSO_2 and 7-H), 5.39 (1H, ddd, $J_{8,9a}$ = 2.7, $J_{8,7}$ = 5.1, $J_{8,9b}$ = 7.4 Hz, 8-H), 4.66 (1H, dd, $J_{9a,8}$ = 2.7, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.36 (1H, ddd, $J_{5,4}$ = 4.6, $J_{5,6}$ = $J_{5,NH}$ = 10.4 Hz, 5-H), 4.22 (1H, dd, $J_{6,7}$ = 2.0, $J_{6,5}$ = 10.4 Hz, 6-H), 4.14 (1H, dd, $J_{9b,8}$ = 7.4, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.88 (1H, m, 4-H), 3.75 (3H, s, $COOCH_3$), 2.09 (3H, s, CH_3COO), 2.08 (3H, s, $COOCH_3$), 2.04 (3H, s, $COOCH_3$) ppm. ^{13}C NMR ($CDCl_3$) 170.8, 169.6 (3C, 3 X $\underline{CO}CH_3$), 161.4 (C-1), 157.4 (q, J_{C-F} = 38 Hz, $\underline{C}OCF_2$), 145.7 (C-2), 144.6-127.4 (6C, Ph), 119.0-110.0 (CF_3), 106.1 (C-3), 72.8 (C-6), 70.5 (C-8), 67.7 (C-7), 62.2 (C-9), 52.6 ($COOCH_3$), 47.1 (C-4), 46.1 (C-5), 20.7, 20.6, 20.3 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 625.2 $[M+H]^+$, 647.3 $[M+Na]^+$, 1271.4 $[2M+Na]^+$. $C_{24}H_{27}F_3N_2O_{12}S$: calcd C, 46.16; H, 4.36; N, 4.49; found C, 45.97; H, 4.21; N, 5.33

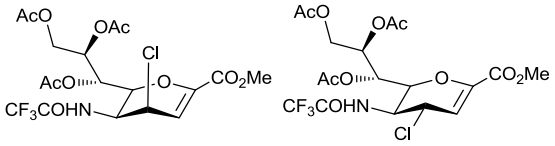
Preparation of methyl 4-(4-methylphenylsulfonamido)-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (38)



38

Starting from glycal **19** (105 mg, 0.2 mmol) and *p*-toluenesulfonamide (342 mg, 2.0 mmol), after 15 min heating at 40 °C in DCM the glycal **38** is obtained as a white solid (109.8 mg, 86%). Glycal **38** shows: m.p. 133-135°C; $[\alpha]_D$ – 21.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.74-7.69 (2H, overlapping, $SO_2\underline{Ph}CH_3$), 7.49 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 7.25-7.30 (2H,overlapping, , $SO_2\underline{Ph}CH_3$), 561 (1H, d, $J_{NH,4}$ = 8.0 Hz, H-NSO_2), 6.09 (1H, d, $J_{3,4}$ = 5.7 Hz, 3-H), 5.48 (1H, dd, $J_{7,6}$ = 2.0, $J_{7,8}$ = 5.1 Hz, 7-H), 5.40 (1H, ddd, $J_{8,9a}$ = 2.7, $J_{8,7}$ = 5.1, $J_{8,9b}$ = 7.4 Hz, 8-H), 4.65 (1H, dd, $J_{9a,8}$ = 2.7, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.34 (1H, ddd, $J_{5,4}$ = 4.6, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.23 (1H, dd, $J_{6,7}$ = 2.0, $J_{6,5}$ = 10.0 Hz, 6-H), 4.13 (1H, dd, $J_{9b,8}$ = 7.4, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.84 (1H, ddd, $J_{4,5}$ = 4.6, $J_{4,3}$ = 5.7, $J_{4,NH}$ = 8.0 Hz, 4-H), 3.74 (3H, s, $COOCH_3$), 2.44 (3H, s, $SO_2Ph\underline{CH_3}$), 2.08 (3H, s, CH_3COO), 2.06 (3H, s, $COOCH_3$), 2.03 (3H, s, $COOCH_3$) ppm. ^{13}C NMR ($CDCl_3$) 170.9, 169.7 (3C, 3 X $\underline{CO}CH_3$), 161.6 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_2$), 145.6 (C-2), 144.6-127.4 (6C, Ph), 119.0-110.0 (CF_3), 106.0 (C-3), 72.8 (C-6), 70.8 (C-8), 67.7 (C-7), 62.2 (C-9), 52.6 ($COOCH_3$), 47.0 (C-4), 46.1 (C-5), 21.6 ($Ph\underline{CH_3}$), 20.8, 20.6, 20.4 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 639.4 $[M+H]^+$, 661.3 $[M+Na]^+$, 1300.0 $[2M+Na]^+$. $C_{25}H_{29}F_3N_2O_{12}S$: calcd C, 40.57; H, 4.48; N, 4.98; found C, 40.88; H, 4.57; N, 5.11;

Preparation of methyl 4-chloro-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-O-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (39) and methyl 4- chloro-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-O-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (40)

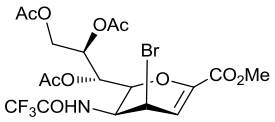


39 **40**

Starting from glycal **19** (105 mg, 0.2 mmol) and TMSCl (254 µL, 2.0 mmol), after 15 min heating at 40 °C in DCM the), a mixture of the glycals **39** and **40** is obtained that, after rapid chromatography, eluting with hexane/AcOEt (80:20 v/v), afforded in the sequence, the less polar glycal **39** (58.9 mg, 19.5%) followed by the more polar glycal **40** (19.6 mg, 58.5%), both in pure form. Glycal **39** shows: m.p. 109-111°C; $[\alpha]_D$ - 19.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.71 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.23 (1H, d, $J_{3,4}$ = 5.6 Hz, 3-H), 5.47 (1H, dd, $J_{7,6}$ = 2.1, $J_{7,8}$ = 5.4 Hz, 7-H), 5.36 (1H, ddd, $J_{8,9a}$ = 2.8, $J_{8,7}$ = 5.4, $J_{8\text{-}9b}$ = 6.4 Hz, 8-H), 4.74 (1H, dd, $J_{4,5}$ = 4.0, $J_{4,3}$ = 5.6 Hz, 4-H), 4.71 (1H, ddd, $J_{5,4}$ = 4.0, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.66 (1H, dd, $J_{9a,8}$ = 2.8, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.52 (1H, dd, $J_{6,7}$ = 2.1, $J_{6,5}$ = 10.0 Hz, 6-H), 4.15 (1H, dd, $J_{9b,8}$ = 6.4, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.83 (3H, s, $COOCH_3$), 2.10 (3H, s, CH_3COO), 2.09 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.1, 169.6 (3C, $COCH_3$), 161.2 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 145.3 (C-2), 119.0-110.0 (CF_3), 107.8 (C-3), 72.6 (C-6), 70.7 (C-8), 67.2 (C-7), 61.8 (C-9), 53.8 (C-4), 52.8 ($COOCH_3$), 46.4 (C-5), 20.8, 20.7, 20.5 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 504.8 $[M+H]^+$, 526.7 $[M+Na]^+$. $C_{18}H_{21}ClF_3NO_{10}$: calcd C, 42.91; H, 4.20; N, 2.78; found C, 42.01; H, 3.98; N, 2.42. Glycal **40** shows: m.p. 115-117°C; $[\alpha]_D$ + 51.2 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.18 (1H, d, $J_{NH,5}$ = 9.0 Hz, N-H), 6.07 (1H, d, $J_{3,4}$ = 2.5 Hz, 3-H), 5.39 (1H, dd, $J_{7,6}$ = 1.6, $J_{7,8}$ = 5.6 Hz, 7-H), 5.31 (1H, ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = $J_{8\text{-}9b}$

= 6.0 Hz, 8-H), 4.99 (1H, dd, $J_{4,3}$ = 2.5, $J_{4,5}$ = 9.9 Hz, 4-H), 4.65 (1H, dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.53 (1H, dd, $J_{6,7}$ = 1.6, $J_{6,5}$ = 9.0 Hz, 6-H), 4.18 (1H, dd, $J_{9b,8}$ = 6.0, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 4.05 (1H, ddd, $J_{5,4}$ = $J_{5,6}$ = $J_{5,NH}$ = 9.0 Hz, 5-H), 3.82 (3H, s, $COOCH_3$), 2.15 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), ppm. ^{13}C NMR ($CDCl_3$) 170.8, 170.5, 170.2 (3C, $COCH_3$), 161.2 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 144.3 (C-2), 119.0-110.0 (CF_3), 110.1 (C-3), 75.3 (C-6), 70.4 (C-8), 67.6 (C-7), 61.7 (C-9), 52.9 (C-4), 52.7 ($COOCH_3$), 52.5 (C-5), 20.8, 20.6 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 504.7 $[M+H]^+$, 526.7 $[M+Na]^+$. $C_{18}H_{21}ClF_3NO_{10}$: calcd C, 42.91; H, 4.20; N, 2.78; found C, 42.11; H, 4.01; N, 2.47.

Preparation of methyl 4-bromo-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (41)

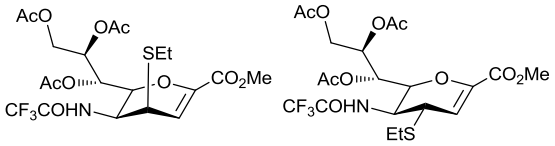


41

Starting from glycal **19** (105 mg, 0.2 mmol) and TMSBr (264 µL, 2.0 mmol), after 30 min heating at 40 °C in DCM the glycal **41** is obtained as a white solid (80.0 mg, 73%). Glycal **41** shows: m.p. 103-105°C; $[\alpha]_D$ - 23.4 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.75 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.31 (1H, d, $J_{3,4}$ = 5.9 Hz, 3-H), 5.47 (1H, dd, $J_{7,6}$ = 2.0, $J_{7,8}$ = 6.0 Hz, 7-H), 5.37 (1H, ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = $J_{8\text{-}9b}$ = 6.0 Hz, 8-H), 4.91 (1H, dd, $J_{4,5}$ = 4.0, $J_{4,3}$ = 5.9 Hz, 4-H), 4.69-4.61 (2H, overlapping, 9a-H and 6-H), 4.51 (1H, ddd, $J_{5,4}$ = 4.0, $J_{5,6}$ = $J_{5,NH}$ = 10.0 Hz, 5-H), 4.15 (1H, dd, $J_{9b,8}$ = 6.0, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.82 (3H, s, $COOCH_3$), 2.10 (3H, s, CH_3COO), 2.09 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.7, 170.1, 169.6 (3C, $COCH_3$), 161.1 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 144.7 (C-2), 119.0-110.0 (CF_3), 109.1 (C-3), 73.4 (C-6), 70.7 (C-8), 67.3 (C-7), 61.9 (C-9), 52.8 ($COOCH_3$), 47.2 (C-4), 45.8 (C-5), 20.8, 20.7, 20.5 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 549.3 $[M+H]^+$, 571.2 $[M+Na]^+$.

$C_{18}H_{21}BrF_3NO_{10}$: calcd C, 39.43; H, 3.86; N, 2.55; found C, 38.88; H, 3.56; N, 2.31.

Preparation of methyl 4-thioethoxy-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (42) and methyl 4- thioethoxy-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (43)

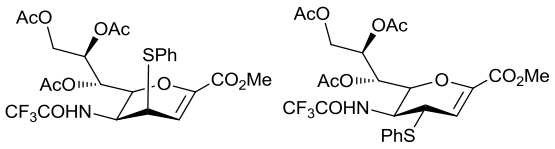


42 **43**

Starting from glycal **19** (105 mg, 0.2 mmol) and EtSH (144 µL, 2.0 mmol), after 15 min heating at 40 °C in DCM the), a mixture of the glycals **42** and **43** is obtained that, after flash chromatography, eluting with hexane/AcOEt (80:20 v/v), afforded in the sequence, the less polar glycal **42** (30.0 mg, 28.3%),followed by the more polar glycal **43** (59.9 mg, 56.6%) both in pure form. Glycal **42** shows: m.p. 133-135°C; $[\alpha]_D$ - 29.9 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 8.12-7.34 (15H, 3 X Ph), 6.93 (1H, d, $J_{NH,5}$ = 9.7 Hz, N-H), 6.21 (1H, d, $J_{3,4}$ = 5.2 Hz, 3-H), 5.99 (1H, dd, $J_{7,6}$ = 2.8, $J_{7,8}$ = 4.4 Hz, 7-H), 5.91 (1H, ddd, $J_{8,9a}$ = 3.0, $J_{8,7}$ = 4.4, $J_{8\text{-}9b}$ = 7.2 Hz, 8-H), 5.22 (1H, dd, $J_{9a,8}$ = 3.0, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.68 (1H, ddd, $J_{5,4}$ = 5.2, $J_{5,6}$ = $J_{5,NH}$ = 9.7 Hz, 5-H), 6.61 (1H, dd, $J_{9b,8}$ = 7.2, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 4.45 (1H, dd, $J_{6,7}$ = 2.8, $J_{6,5}$ = 9.7 Hz, 6-H), 3.78 (3H, s, $COOCH_3$), 3.60 (1H, dd, $J_{4,3}$ = $J_{4,5}$ = 5.2 Hz, 4-H), 2.65-2.58 (2H, m, $S\underline{CH_2}CH_3$), 1.26 (3H, t, $J_{CH3,CH2S}$ = 7.4 Hz, $SCH_2\underline{CH_3}$) ppm. ^{13}C NMR ($CDCl_3$) 170.2, 170.1, 169.6 (3C, $COCH_3$), 161.4 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 143.1 (C-2), 119.0-110.0 (CF_3), 109.6 (C-3), 73.7 (C-6), 70.8 (C-8), 67.6 (C-7), 61.8 (C-9), 52.5 ($COOCH_3$), 44.9 (C-5), 42.3 (C-4), 28.7 ($S\underline{CH_2}CH_3$), 20.8, 20.7, 20.6 (3C, $\underline{CH_3}COO$), 15.1 (SCH_2CH_3) ppm. MS (ESI positive) *m/z* 716.7 $[M+H]^+$, 738.6 $[M+Na]^+$. $C_{35}H_{32}F_3NO_{10}S$: calcd C, 58.74; H, 4.51; N, 1.96; found C, 58.00; H, 4.33; N, 1.81. Glycal **43** shows: m.p. 121-123°C; $[\alpha]_D$ + 33.5 (*c* 1 in $CHCl_3$). ^1H

NMR ($CDCl_3$): δ = 6.70 (1H, d, $J_{NH,5}$ = 9.0 Hz, N-H), 6.13 (1H, d, $J_{3,4}$ = 3.0 Hz, 3-H), 5.45 (1H, dd, $J_{7,6}$ = 2.8, $J_{7,8}$ = 5.8 Hz, 7-H), 5.37 (1H, ddd, $J_{8,9a}$ = 2.9, $J_{8,7}$ = 5.8, $J_{8\text{-}9b}$ = 7.0 Hz, 8-H), 4.63 (1H, dd, $J_{9a,8}$ = 2.9, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.39 (1H, dd, $J_{6,7}$ = 2.8, $J_{6,5}$ = 9.2 Hz, 6-H), 4.19 (1H, dd, $J_{9b,8}$ = 7.0, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.98 (1H, ddd, $J_{5,4}$ = $J_{5,6}$ = $J_{5,NH}$ = 9.0 Hz, 5-H), 3.81 (3H, s, $COOCH_3$), 3.70 (1H, dd, , $J_{4,3}$ = 3.0, $J_{4,5}$ = 9.0 Hz, 4-H), 2.60 (2H, m, $S\underline{CH_2}CH_3$), 2.14 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.3, 170.0 (3C, $COCH_3$), 161.6 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_2$), 144.0 (C-2), 119.0-110.0 (CF_3), 112.1 (C-3), 75.7 (C-6), 70.4 (C-8), 68.0 (C-7), 61.8 (C-9), 52.5 ($COOCH_3$), 49.5 (C-5), 42.1 (C-4), 23.9 ($S\underline{CH_2}CH_3$), 20.8, 20.6, (3C, $\underline{CH_3}COO$), 14.5 (SCH_2CH_3) ppm. MS (ESI positive) *m/z* 530.6 $[M+H]^+$, 552.5 $[M+Na]^+$. $C_{20}H_{26}F_3NO_{10}S$: calc C, 45.37; H, 4.95; N, 2.65; found C, 45.51; H, 5.05; N, 2.77.

Preparation of methyl 4-phenilthio-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (44) and methyl 4-phenylthio-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (45)

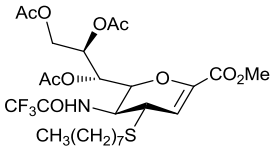


44 **45**

Starting from glycal **19** (105 mg, 0.2 mmol) and PhSH (205 µL, 2.0 mmol), after 15 min heating at 40 °C in DCM the), a mixture of the glycals **44** and **45** is obtained that, after flash chromatography, eluting with hexane/AcOEt (80:20 v/v), afforded in the sequence, the less polar glycal **44** (16.2 mg, 14%),followed by the more polar glycal **45** (80.9 mg, 56.6%) both in pure form. Glycal **44** shows: m.p. 144-146°C; $[\alpha]_D$ – 45.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.41-7.28 (5H, overlapping, S<u>Ph</u>), 6.71 (1H, d, $J_{NH,5}$ = 10.1 Hz, N-H), 6.30 (1H, d, $J_{3,4}$ = 5.6 Hz, 3-H), 5.47 (1H, dd, $J_{7,6}$ = 2.6, $J_{7,8}$ = 5.1 Hz, 7-H), 5.33 (1H, ddd, $J_{8,9a}$ = 3.0, $J_{8,7}$ = 5.1, $J_{8\text{-}9b}$ = 6.7 Hz, 8-H), 4.68-4.62 (2H, overlapping, 9a-H and 5-H), 4.31

(1H, dd, $J_{6,7}$ = 2.6, $J_{6,5}$ = 9.8 Hz, 6-H), 4.19-4.12 (2H, overlapping, 9b-H and 4-H), 3.81 (3H, s, $COOCH_3$), 2.08 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.1, 169.7 (3C, $COCH_3$), 161.5 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 144.0 (C-2), 130.9-128.3 (6C, S$\underline{Ph}$), 119.0-110.0 (CF_3), 108.3 (C-3), 73.6 (C-6), 70.8 (C-8), 67.5 (C-7), 61.9 (C-9), 52.6 ($COOCH_3$), 46.7 (C-5), 45.8 (C-4), 20.9, 20.7, 20.5 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 578.2 $[M+H]^+$, 600.5 $[M+Na]^+$. $C_{24}H_{26}F_3NO_{10}S$: calcd C, 49.91; H, 4.54; N, 2.43; O, 27.70; found C, 49.52; H, 4.41; N, 2.33. Glycal **45** shows: m.p. 111-112°C; $[\alpha]_D$ + 44.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.51-7.46 (2H, overlapping, S$\underline{Ph}$), 7.35-7.29 (3H, overlapping, S$\underline{Ph}$), 7.12 (1H, d, $J_{NH,5}$ = 9.4 Hz, N-H), 6.15 (1H, d, $J_{3,4}$ = 2.7 Hz, 3-H), 5.45 (1H, dd, $J_{7,6}$ = 1.8, $J_{7,8}$ = 5.1 Hz, 7-H), 5.37 (1H, ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = 5.1, $J_{8\text{-}9b}$ = 7.0 Hz, 8-H), 4.68 (1H, dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.32 (1H, dd, $J_{6,7}$ = 1.8, $J_{6,5}$ = 9.4 Hz, 6-H), 4.12 (1H, dd, $J_{9b,8}$ = 7.0, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 4.02 (1H, dd, $J_{4,3}$ = 2.7, $J_{4,5=}$ 9.4 Hz, 4-H), 3.91 (1H, ddd, $J_{5,4}$ = $J_{5,6}$ = $J_{5,NH}$ = 9.4 Hz, 5-H), 3.76 (3H, s, $COOCH_3$), 2.07 (3H, s, CH_3COO), 2.02 (6H, overlapping, 2 X CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.9, 170.5, 170.4 (3C, $COCH_3$), 161.6 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 144.2 (C-2), 134.7-129.1 (6C, S$\underline{Ph}$), 119.0-110.0 (CF_3), 111.9 (C-3), 75.7 (C-6),970.4 (C-8), 67.8 (C-7), 61.9 (C-9), 52.5 ($COOCH_3$), 48.9 (C-4), 46.1 (C-5), 20.8, 20.7, 20.6 (3C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 578.3 $[M+H]^+$, 600.5 $[M+Na]^+$. $C_{24}H_{26}F_3NO_{10}S$: calcd C, 49.91; H, 4.54; N, 2.43; O, 27.70; found C, 50.07; H, 4.63; N, 2.53.

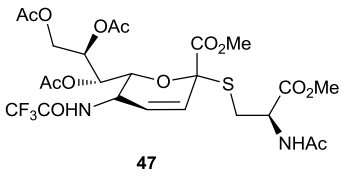
Preparation of methyl 4-thio-*n*-octooxy-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (46)



46

Starting from glycal **19** (105 mg, 0.2 mmol) and $C_8H_{17}SH$ (347 µL, 2.0 mmol), after 30 min heating at 40 °C in DCM the glycal **46** is obtained as a white solid (74.9 mg, 61 %). Glycal **46** shows: m.p. 109-111°C; $[\alpha]_D$ + 46.1 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 6.87 (1H, d, $J_{NH,5}$ = 9.3 Hz, N-H), 6.11 (1H, d, $J_{3,4}$ = 2.9 Hz, 3-H), 5.45 (1H, dd, $J_{7,6}$ = 2.6, $J_{7,8}$ = 5.2 Hz, 7-H), 5.33 (1H, ddd, $J_{8,9a}$ = 2.9, $J_{8,7}$ = 5.2, $J_{8\text{-}9b}$ = 6.7 Hz, 8-H), 4.67 (1H, dd, $J_{9a,8}$ = 2.9, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.37 (1H, dd, $J_{6,7}$ = 2.6, $J_{6,5}$ = 9.3 Hz, 6-H), 4.17 (1H, dd, $J_{9b,8}$ = 6.7, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.99 (1H, ddd, $J_{5,4}$ = $J_{5,6}$ = $J_{5,NH}$ = 9.3 Hz, 5-H), 3.81 (3H, s, $COOCH_3$), 3.66 (1H, dd, $J_{4,3}$ = 2.9, $J_{4,5}$ = 9.3 Hz, 4-H), 2.60-2.47 (2H, m, $S\underline{CH_2}(CH_2)_6CH_3$), 2.13 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 1.54 (2H, m, $SCH_2C\underline{H_2}(CH_2)_5CH_3$), 1.38-1.22 (10H, overlapping, $SCH_2CH_2(\underline{CH_2})_5CH_3$), 0.87 (3H, t, $S(CH_2)_7C\underline{H_3}$) ppm. ^{13}C NMR ($CDCl_3$) 170.8, 170.3, 170.2 (3C, $\underline{C}OCH_3$), 161.7 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_2$), 144.1 (C-2), 119.0-110.0 (CF_3), 112.4 (C-3), 76.0 (C-6), 70.7 (C-8), 68.0 (C-7), 61.9 (C-9), 52.5 ($COO\underline{C}H_3$), 48.8 (C-5), 42.4 (C-4), 31.8 ($S\underline{C}H_2(CH_2)_6CH_3$), 29.6-22.6 (6C, $SCH_2(\underline{CH_2})_6CH_3$), 20.8, 20.7, (3C, $\underline{C}H_3COO$), 14.1 ($S(CH_2)_7\underline{C}H_3$) ppm. MS (ESI positive) *m/z* 614.5 $[M+H]^+$, 636.6 $[M+Na]^+$. $C_{26}H_{38}F_3NO_{10}S$: calcd C, 50.89; H, 6.24; N, 2.28; found C, 50.01; H, 6.13; N, 2.17.

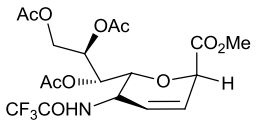
Preparation of Methyl 5-(2,2,2-trifluoroacetamido)-2,6-anhydro-2-[(2-acetamido-methyoxy-3-oxopropyl)thio]-2,3,4,5-tretradeoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enoate (47)



47

Starting from glycal **19** (105 mg, 0.2 mmol) and protected cysteine (71 mg, 0.4 mmol), after 60 min heating at 40 °C in DCM the glycal **47** is obtained as a white solid (101.8 mg, 79 %). Glycal **47** shows: m.p. 141-143°C; $[\alpha]_D$ + 19.4 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.81 (1H, d, $J_{NH,5}$ = 9.6 Hz, N-H), 2.37-6.29 (2H, overlapping, H-3 and CHN<u>H</u>Ac), 5.91 (1H, dd, $J_{4,5}$ = 2.0, $J_{4,3}$ = 10.2 Hz, 4-H), 5.33-5.28 (2H, overlapping, 7-H and 8-H), 4.94 (1H, ddd, $J_{CH,CHa}$ = 3.7, $J_{CH,CHb}$ = $J_{CH,NH}$ = 9.6 Hz, $CH_2C\underline{H}NHAc$), 4.72 (1H, ddd, m, 5-H), 4.55 (1H, dd, $J_{9a,8}$ = 2.3, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.48 (1H, dd, $J_{6,7}$ = 1.8, $J_{6,5}$ = 10.0 Hz, 6-H), 4.19 (1H, dd, $J_{9b,8}$ = 6.2, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.84 (3H, s, $COOCH_3$), 3.78 (3H, s, $COOCH_3$), 3.28 (1H,dd, $J_{CHa,CH}$ = 3.7, $J_{CHa,CHb}$ = 14.4 Hz, $SC\underline{H_a}CH$), 2.84 (1H, dd, $J_{CHb,CH}$ = 9.6, $J_{CHb,CHa}$ = 14.4 Hz, $SC\underline{H_b}CH$), 2.14 (3H, s, CH_3COO), 2.10 (6H, overlapping, 2 X CH_3COO), 2.04 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.8 ($NH\underline{C}OCH_3$), 170.7($\underline{C}OOMe$), 170.7, 170.3, 169.8 (3C, $\underline{C}OCH_3$), 167.7 (C-1), 157.4 (q, J_{C-F} = 38 Hz, $\underline{C}OCF_3$), 130.1 (C-4), 126.4 (C-3), 119.0-110.0 (CF_3), 84.2 (C-2), 70.6 (C-6), 70.2 (C-8), 68.5 (C-7), 62.4 (C-9), 53.3 ($CHCOO\underline{C}H_3$), 52.8 ($COOCH_3$), 51.2 ($\underline{C}HCOOCH_3$), 43.3 (C-5), 33.6 ($S\underline{CH_2}CH$), 23.0 ($\underline{C}H_3CONH$), 21.0, 20.7, 20.5 (3C, $\underline{C}H_3COO$) ppm. MS (ESI positive) *m/z* 645.5 $[M+H]^+$, 668.5 $[M+Na]^+$. $C_{24}H_{31}F_3N_2O_{13}S$: calcd C, 44.72; H, 4.85; N, 4.35; found C, 44.15; H, 4.32; N, 4.09.

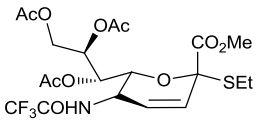
Preparation of Methyl 5-(2,2,2-trifluoroacetamido)-2,6-anhydro-2,3,4,5-tetradeoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (48)



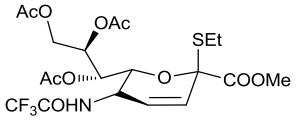
48

Starting from glycal **19** (105 mg, 0.2 mmol) and TESH (319 µL, 2 mmol), after 15 min heating at 40 °C in DCM the glycal **48** is obtained as a white solid (101.8 mg, 79 %). Glycal **48** shows: m.p. 108-110°C; $[\alpha]_D$ + 34.2 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.89 (1H, d, $J_{NH,5}$ = 8.6 Hz, N-H), 6.04 (1H, ddd, $J_{3,5}$ = 2.2, $J_{3,2}$ = 4.3, $J_{3,4}$ = 10.3 Hz, 3-H), 5.82 (1H, ddd, $J_{4,5}$ = 2.5, $J_{4,2}$ = 4.9 , $J_{4,3}$ = 10.3 Hz, 4-H), 5.41 (1H, ddd, $J_{8,9a}$ = 2.3, $J_{8,7}$ = $J_{8\text{-}9b}$ = 6.2 Hz, 8-H), 5.25 (1H, dd, $J_{7,6}$ = 2.3, $J_{7,8}$ = 6.2 Hz, 7-H), 4.80 (1H, m, 2-H), 4.50 (1H, dd, $J_{9a,8}$ = 2.3, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.43 (1H, ddd, m, 5-H), 4.21 (1H, dd, $J_{9b,8}$ = 6.2, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.92 (1H, dd, $J_{6,7}$ = 2.3, $J_{6,5}$ = 10.0 Hz, 6-H), 3.77 (3H, s, $COOCH_3$), 2.13 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.02 (3H, s, CH_3COO) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.1 (3C, $\underline{C}OCH_3$), 168.4 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $\underline{C}OCF_3$), 127.6 (C-4), 126.3 (C-3), 119.0-110.0 (CF_3), 74.8 (C-2), 73.8 (C-6), 70.1 (C-8), 68.4 (C-7), 62.3 (C-9), 52.6 ($COOCH_3$), 44.6 (C-5), 20.8, 20.6 (3C, $\underline{C}H_3COO$) ppm. MS (ESI positive) *m/z* 470.4 $[M+H]^+$, 492.3 $[M+Na]^+$. $C_{18}H_{22}F_3NO_{10}$: calcd C, 45.29; H, 4.80; N, 3.77; found C, 44.88; H, 4.22; N, 3.42.

Preparation of methyl 5-(2,2,2-trifluoroacetamido)-2,6-anhydro-2-ethylthio-2,3,4,5-tetradeoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (49) and methyl 5-(2,2,2-trifluoroacetamido)-2,6-anhydro-2-ethylthio-3,4,5-trideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (50)



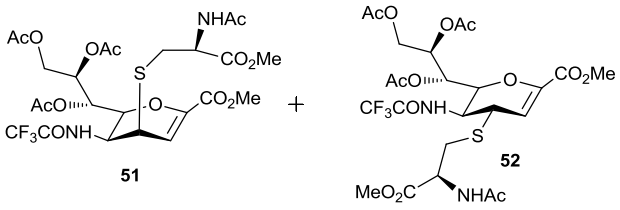
49



50

Starting from glycal **19** (105 mg, 0.2 mmol), EtSH (144 µL, 2 mmol) and BF_3Et_2O (24.6 µL, 0.2 mmol) after 15 min at 25 °C in DCM we obtain a mixture of glycals **42**, **43**, **49** and **50.** the **49-50** inseparable mixture shows: ^{1}H NMR ($CDCl_3$): δ = 6.45 (1H, d, $J_{NH',5'}$ = 8.1 Hz, N-H'), 6.50 (1H, br s, N-H), 6.22 (1H, dd, $J_{3,5}$ = 2.1, $J_{3,4}$ = 10.1 Hz, 3-H), 6.04 (1H, dd, $J_{3',5'}$ = 2.6, $J_{3',4'}$ = 10.1 Hz, 3-H'), 5.90 (1H, dd, $J_{4',5'}$ = 1.8, $J_{4',3'}$ = 10.1 Hz, 4-H'), 5.80 (1H, dd, $J_{4,5}$ = 1.4, $J_{4,3}$ = 10.1 Hz, 4-H), 5.45 (1H, ddd, $J_{8',9a'}$ = 2.5, $J_{8',7'}$ = 4.8, $J_{8'\text{-}9b'}$ = 7.3 Hz, 8'-H), 5.33-5.23 (3H, overlapping, 7-H, 8-H and 7'-H), 4.55 (1H, dd, $J_{9a,8}$ = 2.4, $J_{9a,9b}$ = 12.6 Hz, 9a-H), 4.52-4.47 (3H, overlapping, 9a-H', 5-H and 6-H), 4.32-4.25 (3H, m, 9b-H, 9b-H'and 5-H'), 4.08 (1H, dd, $J_{6',7'}$ = 1.8, $J_{6',5'}$ = 9.8 Hz, 6-H'), 3.83 (3H, s, $COOCH_3$), 3.78 (3H, s, $COOCH_3$'), 4.76-4.66 (2H, m, $S\underline{CH_2}'CH_3$'), 2.66-2.55 (2H, m, $S\underline{CH_2}CH_3$), 2.15 (6H, overlapping, $CH_3'COO$ and CH_3COO), 2.13 (3H, s, $CH_3'COO$), 2.11 (3H, s, CH_3COO), 2.04 (6H, overlapping, $CH_3'COO$ and CH_3COO), 1.26 (3H, m, $SCH_2'CH_3'$), 1.18 (3H, m, SCH_2CH_3) ppm.

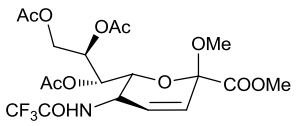
Preparation of methyl 4-[(2-acetamido-methyoxy-3-oxopropyl)thio]-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (51) and methyl 4-[(2-acetamido-methyoxy-3-oxopropyl)thio]-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (52)



Starting from glycal **47** (50 mg, 0.08 mmol) and BF_3Et_2O (492 µL, 4.0 mmol), after 120 min heating at 40 °C in DCM mixture of the glycals **51** and **52** is obtained that, after flash chromatography, eluting with hexane/AcOEt (50:50 v/v), afforded in the sequence, the less polar glycal **51** (27.3 mg, 53.3%),followed by the more polar glycal **52** (13.7 mg, 26.6%) both in pure form. Glycal **51** shows: m.p. 156-158°C; $[\alpha]_D^2$– 38.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.75 (1H, d, $J_{NH,5}$ = 10.0 Hz, N-H), 6.33 (1H, d, $J_{NH,CH}$ = 7.6 Hz, CHN<u>H</u>Ac), 6.10 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 5.48 (1H, dd, $J_{7,6}$ = 2.8, $J_{7,8}$ = 4.8 Hz, 7-H), 5.35 (1H, ddd, $J_{8,9a}$ = 3.1, $J_{8,7}$ = 4.8, $J_{8\text{-}9b}$ = 6.9 Hz, 8-H), 4.84 (1H, ddd, $J_{CH,CHa}$ = 4.9, $J_{CH,CHb}$ = 6.3, $J_{CH,NH}$ = 7.6 Hz, $CH_2C\underline{H}NHAc$), 4.66-4.58 (2H, overlapping, 9a-H, 5-H), 4.26 (1H, dd, $J_{6,7}$ = 2.8, $J_{6,5}$ = 10.0 Hz, 6-H), 4.17 (1H, dd, $J_{9b,8}$ = 6.9, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.80 (6H, overlapping, 2 X $COOCH_3$), 3.66 (1H, dd, , $J_{4,3}$ = $J_{4,5}$ = 5.3 Hz, 4-H), 3.14 (1H,dd, $J_{CHa,CH}$ = 4.8, $J_{CHa,CHb}$ = 14.1 Hz, SC<u>Ha</u>CH), 2.97 (1H, dd, $J_{CHb,CH}$ = 6.3, $J_{CHb,CHa}$ = 14.1 Hz, SC<u>Hb</u>CH), 2.09 (3H, s, CH_3COO), 2.08 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.06 (3H, s, $C\underline{H_3}CONH$) ppm. ^{13}C NMR ($CDCl_3$) 170.6 (2C, NH<u>C</u>OCH_3 and <u>C</u>OOMe), 170.4, 170.1, 169.7 (3C, <u>C</u>OCH_3), 161.5 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, <u>C</u>OCF_3), 143.4 (C-2), 119.0-110.0 (CF_3), 108.4 (C-3), 73.5 (C-6), 70.7 (C-8), 67.8 (C-7), 61.9 (C-9), 53.0 (CHCOO<u>C</u>H_3), 52.6 ($COOCH_3$), 52.4 (<u>C</u>H$COOCH_3$), 46.4 (C-5), 44.0 (C-4), 36.9 (S<u>C</u>H_2CH), 23.0 (<u>C</u>H_3CONH), 20.9, 20.7, 20.5 (3C, <u>C</u>H_3COO) ppm. MS (ESI positive) *m/z* 645.6 $[M+H]^+$, 668.5 $[M+Na]^+$. $C_{24}H_{31}F_3N_2O_{13}S$: calcd C, 44.72; H,

4.85; N, 4.35; found C, 45.05; H, 4.44; N, 4.02. Glycal **52** shows: m.p. 160-162°C; $[\alpha]_D$+ 42.7 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 7.68 (1H, br s, N-H), 6.33 (1H, d, $J_{NH,CH}$ = 7.6 Hz, CHNHAc), 6.04 (1H, d, $J_{3,4}$ = 1.8 Hz, 3-H), 5.45 (1H, dd, $J_{7,6}$ = 2.3, $J_{7,8}$ = 5.9 Hz, 7-H), 5.37 (1H, ddd, $J_{8,9a}$ = 2.7, $J_{8,7}$ = $J_{8\text{-}9b}$ = 5.9 Hz, 8-H), 4.76 (1H, ddd, $J_{CH,CHa}$ = 4.8, $J_{CH,CHb}$ = $J_{CH,NH}$ = 7.6 Hz, CH_2CHNHAc), 4.60-4.53 (2H, overlapping, 9a-H, 6-H), 4.18 (1H, dd, $J_{9b,8}$ = 5.9, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.89-3.83 (2H, overlapping, 5-H and 4-H), 3.82 (3H, s, $COOCH_3$), 3.77 (3H, s, $COOCH_3$), 3.03 (1H,dd, $J_{CHa,CH}$ = 4.8, $J_{CHa,CHb}$ = 14.2 Hz, SCH_aCH), 2.97 (1H, dd, $J_{CHb,CH}$ = 7.6, $J_{CHb,CHa}$ = 14.2 Hz, SCH_bCH), 2.13 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.05 (3H, s, CH_3CONH) ppm. ^{13}C NMR ($CDCl_3$) 170.9 ($NHCOCH_3$) , 170.8 (COOMe), 170.7, 170.1, 170.0 (3C, $COCH_3$), 161.6 (C-1), 157.4 (q, $J_{C\text{-}F}$ = 38 Hz, $COCF_3$), 145.5 (C-2), 119.0-110.0 (CF_3), 111.2 (C-3), 75.9 (C-6), 70.2 (C-8), 67.7 (C-7), 61.9 (C-9), 53.0 ($CHCOOCH_3$), 52.6 ($COOCH_3$), 51.9 ($CHCOOCH_3$), 49.9 (C-5), 42.6 (C-4), 31.8 (SCH_2CH), 23.1 (CH_3CONH), 20.9, 20.7, 20.6 (3C, CH_3COO) ppm. MS (ESI positive) *m/z* 645.6 $[M+H]^+$, 668.5 $[M+Na]^+$. $C_{24}H_{31}F_3N_2O_{13}S$: calcd C, 44.72; H, 4.85; N, 4.35; found C, 45.11; H, 4.38; N, 4.07.

Preparation of Methyl 5-(2,2,2-trifluoroacetamido)-2,6-anhydro-2-methyl-3,4,5-trideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (53)

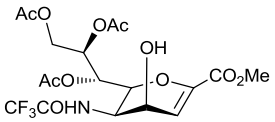


53

Starting from oxazoline **16** (100 mg, 0.24 mmol) is reacted with $Bi(OTf)_3$/Mont-K10 following the procedure of Ikeda et al.[76]. the obtained methylketoside is *N*-translacylated in MeCN with TFAA[81]. The compound **53** is obtained as a white solid (74.9 mg, 61 %). Glycal **53** shows: m.p. 111-112°C; $[\alpha]_D^{20}$ – 36.1 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 6.12 (1H, d, $J_{NH,5}$ = 8.6 Hz, N-H), 5.91 (2H, overlapping, 3-H and 4-H), 5.39 (1H, dd, $J_{7,6}$ = 2.4, $J_{7,8}$ = 4.2 Hz, 7-H), 5.32 (1H, ddd, $J_{8,9a}$ = 2.4, $J_{8,7}$ = $J_{8\text{-}9b}$ = 6.1 Hz, 8-H), 4.66-4.59 (2H, overlapping, 9a-H and 5-

H), 4.24 (1H, dd, $J_{9b,8}$ = 6.1, $J_{9b,9a}$ = 12.6 Hz, 9b-H), 4.05 (1H, dd, $J_{6,7}$ = 2.3, $J_{6,5}$ = 10.3 Hz, 6-H), 3.81 (3H, s, $COOCH_3$), 3.29 (3H, s,OCH_3), 2.15 (3H, s, CH_3COO), 2.09 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 1.98 (3H, s, CH_3CONH) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.2, 169.7 (3C, $COCH_3$), 161.8 (C-1), 157.4 (q, J_{C-F} = 38 Hz, $\underline{C}OCF_3$), 133.2 (C-4), 126.2 (C-3),119.0-110.0 (CF_3), 96.0 (C-2), 71.4 (C-8), 70.3 (C-6), 68.6 (C-7), 62.2 (OCH_3), 62.4 (C-9), 52.6 ($COOCH_3$), 43.9 (C-5), 20.9, 20.7, 20.5 (3C, $\underline{C}H_3COO$) ppm. MS (ESI positive) *m/z* 446.4 $[M+H]^+$, 5468.4 $[M+Na]^+$. $C_{19}H_{27}NO_{11}$: C, 51.23; H, 6.11; N, 3.14; found C, 50.06; H, 5.92 N, 3.03.

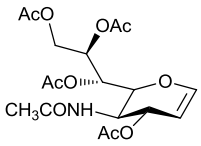
Preparation of Methyl 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (54)



54

Starting from glycal **53** (100 mg, 0.2 mmol) and MeOH (81 µL, 2 mmol), after 15 min heating at 40 °C in DCM the glycal **54** is obtained as a white solid (101.8 mg, 79 %). Glycal **54** shows: m.p. 121-123°C; $[\alpha]_D$ – 49.2 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$) δ = 7.02 (1H, d, $J_{NH,5}$ = 7.4 Hz, $NHCOCF_3$), 6.18 (1H, d, $J_{3,4}$ = 5.5 Hz, 3-H), 5.41 (1H, dd, $J_{7,6}$ = 1.5, $J_{7,8}$ = 4.6 Hz, 7-H), 5.32 (1H, ddd, $J_{8\text{-}9a}$ = 2.7, $J_{8,7}$ = 4.6, $J_{8,9b}$ = 7.3 Hz, 8-H), 4.73 (1H , dd, $J_{9a,8}$ = 2.7, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.38-4.34 (2H, overlapping, 6-H and 5-H), 4.31 (1H, br s, 4-H), 4.16 (1H, dd, $J_{9b,8}$ = 7.3, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.82 (3H, s, $COOCH_3$), 2.80 (1H, br s, OH at C-4), 2.11 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 2.06 (3H, s, O $COCH_3$) ppm. ^{13}C NMR ($CDCl_3$) δ = 170.7, 169.8 (3C, CH_3COO), 161.8 (C-1), 157.1 (q, J_{C-F} = 26 Hz, $COCF_2$), 145.7 (C-2), 119.2-116.0 (1C,CF_3), 108.5 (C-3), 72.5 (C-6), 71.6 (C-8), 67.6 (C-7), 62.1 (C-9), 61.2 (C-4), 52.7 ($COOCH_3$), 47.0 (C-5), 20.9, 20.7, 20.6 (3C, CH_3COO) ppm. MS (ESI positive) *m/z* 486.5 $[M+H]^+$, 508.5 $[M+Na]^+$, 1193.9 $[2M+Na]^+$; (ESI negative) *m/z* 484.2 $[M-H]^-$. $C_{18}H_{22}F_3NO_{11}$: calcd C, 44.54; H, 4.57; N, 2.89; found C, 44.11; H, 4.23; N, 2.76.

Preparation of 3-Acetamido-4-acetoxy-2-(1,2,3-triacetoxy)propyl-3,4,5,6-tetrahydro-2,3-dihydro-2H-pyran (55)

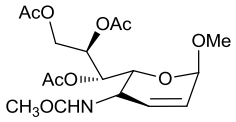


55

Under an atmosphere of nitrogen, a suspension of *N*-acetylneuraminic acid (5 g, 16.2 mmol) in pyridine (75 mL) and acetic anhydride (75 mL) is stirred at room temperature for 12 h and then heated at 100°C for 5 h. The reaction mixture is cooled to room temperature and concentrated under reduced pressure. The residual brownish glassy oil is dissolved in DCM (150 mL) and washed successively with saturated aqueous $NaHCO_3$ (100 mL), aqueous 1 M HCl (100 mL), and brine (100 mL). The organic layer is dried over $MgSO_4$, filtered, and concentrated. The brownish residue is purified by column chromatography on silica gel (EtOAc/hexane, 67:33 to 100:0) to afford protected decarboxylate *N*-acetylneuraminic acid as a pale-yellow foam (3.8 g, 50%)[107]. This compound is then 1,2 unsaturated, introducing a chlorine atom at C-1 using AcCl and MeOH and eliminated under basic conditions of Na_2HPO_4 in MeCN to afford glycal **55** as white solid (2.7 g, 80%). Glycal **55** shows: m.p. 101-103°C; $[\alpha]_D$ + 42.9 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.42 (1H, d, $J_{2,3}$ = 6.1 Hz, 2-H), 5.62 (1H, d, $J_{NH,5}$ = 9.0 Hz, N-H), 5.45 (1H, dd, $J_{7,6}$ = 3.4, $J_{7,8}$ = 5.5 Hz, 7-H), 5.38 (1H, dd,, $J_{4,3}$ = 2.5, $J_{4,5}$ = 9.0 Hz, 4-H), 5.33 (1H, ddd, $J_{8,9a}$ = 3.1, $J_{8,7}$ = 5.5, $J_{8\text{-}9b}$ = 6.0 Hz, 8-H), 4.80 (1H, d, $J_{3,4}$ = 2.5, $J_{3,2}$ = 6.1 Hz, 3-H), 4.40 (1H, dd, $J_{9a,8}$ = 3.1, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.35 (1H, ddd, $J_{5,4}$ = $J_{5,6}$ = $J_{5,NH}$ = 9.0 Hz, 5-H), 4.21 (1H, dd, $J_{6,7}$ = 3.4, $J_{6,5}$ = 9.0 Hz, 6-H), 4.12 (1H, dd, $J_{9b,8}$ = 6.0, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 2.11 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.05-2.03 (6H, overlapping, 2 X CH_3COO), 1.92 (3H, s, $NHCOCH_3$) ppm. ^{13}C NMR ($CDCl_3$) 171.1 ($HN\underline{C}OCH_3$), 170.6, 170.1, 169.9 (4C, $\underline{C}OCH_3$), 145.6 (C-2), 100.3 (C-3), 75.4 (C-6), 69.9 (C-8), 68.5 (C-4), 67.7 (C-7), 61.9 (C-9), 47.2 (C-5), 23.2 ($\underline{C}H_3CONH$), 21.0, 20.8, 20.7, (4C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 416.4

[M+H]$^+$, 438.5 [M+Na]$^+$. $C_{18}H_{25}NO_{10}$: calc C, 52.05; H, 6.07; N, 3.37; found C, 52.48; H, 6.31; N, 3.51.

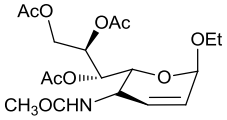
Preparation of 3-acetamido-6-methoxy-2-(1,2,3-triacetoxy)propyl-3,4,5,6-tetrahydro-4,5-dihydro-2H-pyran (56)



56

Starting from glycal **55** (85 mg, 0.2 mmol) and MeOH (81 µL, 2 mmol), after 15 min heating at 40 °C in DCM the glycal **56** is obtained as a white solid (62.2 mg, 76 %). Glycal **56** shows: m.p. 97-99°C; $[\alpha]_D$ + 19.0 (*c* 1 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 5.80 (1H, d app, $J_{3,4}$ = 10.1 Hz, 3-H), 5.74 (1H, ddd, $J_{4,5}$ = 2.5, $J_{4,2}$ = 5.0, $J_{4,3}$ = 10.1 Hz, 4-H), 5.41-5.30 (3H, overlapping, N-H, 7-H and 8-H), 4.93 (1H, br s, 2-H), 4.59 (1H, m, 5-H), 4.47 (1H, dd, $J_{9a,8}$ = 2.5, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.16 (1H, dd, $J_{9b,8}$ = 5.7, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.93 (1H, dd, $J_{6,7}$ = 1.5, $J_{6,5}$ = 10.2 Hz, 6-H), 3.40 (3H, s, OCH_3), 2.14 (3H, s, CH_3COO), 2.10 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 1.98 (3H, s, $NHCOCH_3$) ppm. ^{13}C NMR ($CDCl_3$) 170.7 ($HN\underline{C}OCH_3$), 170.0, 169.9, 169.7 (3C, $\underline{C}OCH_3$), 131.5 (C-3), 126.0 (C-4), 94.2 (C-2), 70.5 (C-7), 68.3 (C-6), 68.2 (C-8), 65.0 (OCH_3), 62.0 (C-9), 43.4 (C-5), 23.4 ($\underline{C}H_3CONH$), 21.0, 20.8, 20.7, (4C, $\underline{CH_3}COO$) ppm. MS (ESI positive) *m/z* 388.4 [M+H]$^+$, 411.4 [M+Na]$^+$. $C_{17}H_{25}NO_9$: calc C, 52.71; H, 6.50; N, 3.62; found C, 52.00; H, 6.29; N, 3.44.

Preparation of 3-acetamido-6-ethoxy-2-(1,2,3-triacetoxy)propyl-3,4,5,6-tetrahydro-4,5-dihydro-2H-pyran (57)

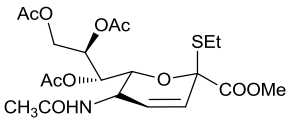


57

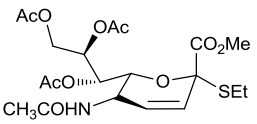
Starting from glycal **55** (85 mg, 0.2 mmol) and EtOH (117 µL, 2 mmol), after 15 min heating at 40 °C in DCM the glycal **57** is obtained as a white solid (61.8

mg, 73 %). Glycal **57** shows: m.p. 88-90°C; $[\alpha]_D$ + 39.4 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 5.79 (1H, d app, $J_{3,4}$ = 10.2 Hz, 3-H), 5.75 (1H, ddd, $J_{4,5}$ = 2.4, $J_{4,2}$ = 4.9, $J_{4,3}$ = 10.2 Hz, 4-H), 5.37-5.27 (3H, overlapping, N-H, 7-H and 8-H), 5.05 (1H, br s, 2-H), 4.60 (1H, m, 5-H), 4.46 (1H, dd, $J_{9a,8}$ = 2.4, $J_{9a,9b}$ = 12.5 Hz, 9a-H), 4.16 (1H, dd, $J_{9b,8}$ = 5.8, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.94 (1H, dd, $J_{6,7}$ = 1.8, $J_{6,5}$ = 10.2 Hz, 6-H), 3.77 (1H, m, $CHaCH_3$), 3.50 (1H, m, $CHbCH_3$), 2.14 (3H, s, CH_3COO), 2.10 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.98 (3H, s, $NHCOCH_3$), 1.19 (3H, t, $J_{CH3,CH2}$ = 7.1 Hz, CH_2CH_3) ppm. ^{13}C NMR ($CDCl_3$) 170.7 ($HN\underline{C}OCH_3$), 170.1, 169.9, 169.8 (3C, $\underline{C}OCH_3$), 131.9 (C-3), 126.3 (C-4), 94.6 (C-2), 70.1 (C-7), 68.3 (C-6), 68.0 (C-8), 64.7 ($\underline{C}H_2CH_3$), 62.2 (C-9), 43.4 (C-5), 23.4 ($\underline{C}H_3CONH$), 21.0, 20.8, 20.7, (4C, $\underline{C}H_3COO$), 15.1 ($CH_2\underline{C}H_3$) ppm. MS (ESI positive) *m/z* 402.4 $[M+H]^+$, 424.4 $[M+Na]^+$. $C_{18}H_{27}NO_9$: calc C, 53.86; H, 6.78; N, 3.49; found C, 53.21; H, 6.39; N, 3.20.

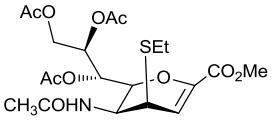
Preparation of Methyl 5-acetamido-2,6-anhydro-2β-ethylthio-2,3,4,5-tetradeoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (58), methyl 5-acetamido-2,6-anhydro-2α-ethylthio-2,3,4,5-tetradeoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (59), methyl 4-ethylthio-2,6-anhydro-5-acetamido-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*talo*-non-2-enonate (60) and methyl 4- ethylthio-2,6-anhydro-5-(2,2,2-trifluoroacetamido)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (61)



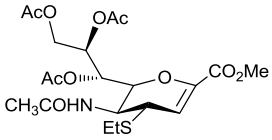
58



59



60



61

Starting from glycal **15** (50 mg, 0.08 mmol) and BF_3Et_2O (492 µL, 4.0 mmol), after 15 min heating at 40 °C in DCM mixture of the glycals **58**, **59**, **60** and **61** is obtained that, after flash chromatography, eluting with hexane/AcOEt 25:75 v/v), afforded in the sequence, the less polar glycal **59** (27.3 mg, 53.3%),followed by the more polar glycal **58** (13.7 mg, 26.6%), both in pure

form and, finally, the mixture of **60** and **61**. Glycal **58** shows: m.p. 140-142°C; $[\alpha]_D$ -36.4 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 5.97 (1H, dd, $J_{3,5}$ = 2.5, $J_{3,4}$ = 10.1 Hz, 3-H), 5.89 (1H, dd, $J_{4,5}$ = 1.7, $J_{4,3}$ = 10.1 Hz, 4-H), 5.46 (1H, ddd, $J_{8,9a}$ = 2.5, $J_{8\text{-}9b}$ = 5.6, $J_{8,7}$ = 6.3 Hz, 8-H), 5.43 (1H, d, $J_{NH,5}$ = 9.1 Hz, N-H), 5.38 (1H, dd, $J_{7,6}$ = 2.0, $J_{7,8}$ = 6.3 Hz, 7-H), 4.57 (1H, dd, $J_{9a,8}$ = 2.5, $J_{9a\text{-}9b}$ = 12.5 Hz, 9a-H), 4.48 (1H, m, 5-H), 4.28 (1H, dd, $J_{9b,8}$ = 5.6, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 3.97 (1H, dd, $J_{6,7}$ = 2.0, $J_{6,5}$ = 9.8 Hz, 6-H), 3.79 (3H, s, $COOCH_3$), 2.77-2.68 (2H, overlapping, SCH_2CH_3), 2.16 (3H, s, CH_3COO), 2.14 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 1.99 (3H, s, CH_3CONH), 1.28 (3H, t, SCH_2CH_3) ppm; ^{13}C NMR ($CDCl_3$) 170.5 (1C, CH_3CONH), 170.4, 170.2, 169.8 (3C, $COCH_3$), 167.3 (C-1), 130.7 (C-4), 126.2 (C-3), 85.7 (C-2), 71.4 (C-8), 70.7 (C-6), 68.5 (C-7), 62.5 (C-9), 52.8 ($COOCH_3$), 43.1 (C-5), 24.4 (1C, SCH_2CH_3), 23.3 (1C, CH_3CONH), 21.0, 20.7 (3C, CH_3COO), 14.1 (SCH_2CH_3) ppm. MS (ESI positive) *m/z* 476.1 $[M+H]^+$, 498.5 $[M+Na]^+$. $C_{20}H_{29}NO_{10}S$: C, 50.52; H, 6.15; N, 2.95; found C, 49.91; H, 6.03; N, 2.50. Compound **59** shows: m.p. 147-149°C; $[\alpha]_D^{20}$ + 25.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.10 (1H, dd, $J_{3,5}$ = 2.2, $J_{3,4}$ = 10.1 Hz, 3-H), 5.74 (1H, dd, $J_{4,5}$ = 1.3 , $J_{4,3}$ = 10.1 Hz, 4-H), 5.59 (1H, d, $J_{NH,5}$ = 9.5 Hz, N-H), 5.38 (1H, dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 3.6 Hz, 7-H), 5.22 (1H, m, 8-H), 4.66-4.58 (2H, overlapping, 9a-H and 5-H), 4.33 (1H, dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.0 Hz, 6-H), 4.24 (1H, dd, $J_{9b,8}$ = 6.8, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 2.64-2.53 (2H, m, $CH_3\underline{CH_2}S$), 2.13 (3H, s, CH_3COO), 2.08 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.96 (3H, s, CH_3CONH), 1.16 (3H, t, $SCH_2C\underline{H_3}$) ppm. ^{13}C NMR ($CDCl_3$) 170.5 ($CH_3\underline{C}ONH$), 170.4, 170.2, 169.8 (3C, $\underline{C}OCH_3$), 167.3 (C-1), 130.7 (C-4), 126.2 (C-3), 85.7 (C-2), 71.4 (C-8), 70.7 (C-6), 68.5 (C-7), 62.5 (C-9), 52.8 ($COOCH_3$), 43.1 (C-5), 24.4 ($CH_3\underline{C}H_2S$), 23.3 ($\underline{C}H_3CONH$), 21.0, 20.7 (3C, $\underline{C}H_3COO$), 14.1 ($\underline{C}H_3CH_2S$) ppm. MS (ESI positive) *m/z* 476.1 $[M+H]^+$, 498.5 $[M+Na]^+$. $C_{20}H_{29}NO_{10}S$: calcd C, 50.52; H, 6.15; N, 2.95 %; found C, 49.91; H, 6.03; N, 2.50. the **60-61** inseparable mixture shows: ^1H NMR ($CDCl_3$): δ = 6.17 (1H, d, $J_{3,4}$ = 5.3 Hz, 3-H), 6.12 (1H, dd, $J_{3',4'}$ = 3.0 Hz, 3-H'), 6.88 (1H, d, $J_{NH,5}$ = 9.2 Hz, N-H), 5.57 (1H, d, $J_{NH,5}$ = 9.9 Hz, N-H), 5.52 (1H, dd, $J_{7',6'}$ = 3.0, $J_{7',8'}$ = 4.9 Hz, 7'-H), 5.49 (1H, dd, $J_{7,6}$ = 2.6, $J_{7,8}$

= 4.5 Hz, 7′-H), 5.36 (1H, ddd, $J_{8',9a'}$ = 3.0, $J_{8',7'}$ = 4.9, $J_{8'\text{-}9b'}$ = 7.0 Hz, 8′-H), 5.31 (1H, ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = 4.5, $J_{8\text{-}9b}$ = 7.1 Hz, 8-H), 4.71 (1H, dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.4 Hz, 9a-H), 4.73 (1H, dd, $J_{9a',8'}$ = 3.0, $J_{9a',9b'}$ = 12.4 Hz, 9a′-H), 4.36-4.3 (2H, overlapping, (6-H and 6′-H), 4.24 (1H, dd, $J_{5,4}$ = 5.6, $J_{5,6}$ = $J_{5,NH}$ = 9.9 Hz, 5-H), 4.22-4.15 (2H, overlapping, 9b-H and 9b′-H), 3.96 (1H, $J_{5',4'}$ = $J_{5',6'}$ = $J_{5',NH'}$ = 9.2 Hz, 5′-H), 3.82 (3H, s, $COOCH_3$), 3.79 (3H, s, $COOCH_3$′), 3.60 (1H, $J_{4',3'}$ = 3.0, $J_{4',5'}$ = 9.2 Hz, 4′-H), 3.50 (1H, $J_{4,3}$ = 5.3, $J_{4,5}$ = 9.9 Hz, 4′-H), 2.67-2.53 (4H, overlapping, $SCH_2'CH_3'$ and SCH_2CH_3), 2.13 (6H, overlapping, $CH_3'COO$ and CH_3COO), 2.07-2.03 (12H, overlapping, 2 x $CH_3'COO$ and 2 x CH_3COO), 1.99 (6H, overlapping, $CH_3'COO$ and CH_3COO), 1.28 (3H, m, $SCH_2'CH_3'$), 1.24 (3H, m, SCH_2CH_3) ppm.

5.3.5 General protocol to synthesize new 3,4 unsaturated *N*-acetyl neuraminic acid conjugates and their possible derivatizations

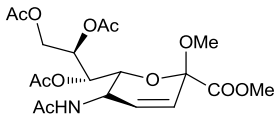
Preparation of Al^{3+}-mont catalyst:

A mixture of parent Na^+-mont (4.2 g) and 200 mL of aqueous $AlCl_3 \cdot 6H_2O$ (3.3×10^{-2} M) is stirred at 50˚C for 24 h. The slurry obtained is filtered and washed with 1 L of distilled water, followed by drying at 110°C in air to afford the Al^{3+}-mont as a whitish gray powder, as suggested by Kaneda et al.[108]

General protocol

The oxazoline **16** (85 mg, 0.2 mmol) is treated with Al^{3+}-mont catalyst (8.5 mg, 10 % w/w) with the appropriate alchol or thiol (2.0 mmol). Then the catalyst is filtered, the solvent is evaporated under reduced pressure, and the residue is purified by flash chromatography.

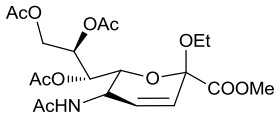
Preparation of methyl 5-acetamido-2,6-anhydro-2-methyl-3,4,5-trideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (62a)



62a

Starting from the oxazoline **16** with MeOH (81 µL, 2.0 mmol) the compound **62a** is obtained after 1 hours at 50 °C, as a white solid (86.4 mg, 97%). Compound **62a** shows: m.p. 118-121°C; $[\alpha]_D$ – 37.9 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 5.91 (2H, overlapping, 3-H and 4-H), 5.43-5.37 (2H, d, overlapping, N-H and 7-H), 5.32 (1H, ddd, $J_{8,9a}$ = 2.4, $J_{8,7}$ = $J_{8\text{-}9b}$ = 6.1 Hz, 8-H), 4.66-4.59 (2H, overlapping, 9a-H and 5-H), 4.24 (1H, dd, $J_{9b,8}$ = 6.1, $J_{9b,9a}$ = 12.6 Hz, 9b-H), 4.05 (1H, dd, $J_{6,7}$ = 2.3, $J_{6,5}$ = 10.3 Hz, 6-H), 3.81 (3H, s, $COOCH_3$), 3.29 (3H, s,OCH_3), 2.15 (3H, s, CH_3COO), 2.09 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 1.98 (3H, s, CH_3CONH) ppm. ^{13}C NMR ($CDCl_3$) 170.6, 170.2, 169.7 (3C, $COCH_3$), 161.8 (C-1), 133.2 (C-4), 126.2 (C-3), 96.0 (C-2), 71.8 (C-8), 70.8 (C-6), 68.7 (C-7), 62.9 (OCH_3), 62.5 (C-9), 52.7 ($COOCH_3$), 43.6 (C-5), 23.2 ($\underline{C}H_3CONH$), 20.9, 20.7, 20.5 (3C, $\underline{C}H_3COO$) ppm. MS (ESI positive) *m/z* 446.4 $[M+H]^+$, 5468.4 $[M+Na]^+$. $C_{19}H_{27}NO_{11}$: C, 51.23; H, 6.11; N, 3.14; found C, 50.06; H, 5.92 N, 3.03.

Preparation of methyl 5-acetamido-2,6-anhydro-2-ethyl-3,4,5-trideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (63)

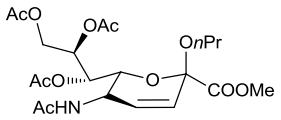


63

Starting from the oxazoline **16** with EtOH (117 µL, 2.0 mmol) the compound **63** is obtained after 1 hours at 50 °C, as a white solid (85.4 mg, 93%). Compound **63** shows: m.p. 132-134°C; $[\alpha]_D$ – 49.5 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 5.97 (2H, overlapping, 3-H and 4-H), 5.43-5.37 (2H, d, overlapping, N-H and 7-H),

5.30 (1H, ddd, $J_{8,9a}$ = 2.4, $J_{8,7}$ = $J_{8\text{-}9b}$ = 6.1 Hz, 8-H), 4.66-4.59 (2H, overlapping, 9a-H and 5-H), 4.23 (1H, dd, $J_{9b,8}$ = 6.1, $J_{9b,9a}$ = 12.6 Hz, 9b-H), 4.02 (1H, dd, $J_{6,7}$ = 2.3, $J_{6,5}$ = 10.3 Hz, 6-H), 3.82 (3H, s, $COOCH_3$), 3.76 (1H, m, OCH_2CH_3), 3.50 (1H, m, OCH_2CH_3), 2.10 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.98 (3H, s, CH_3CONH), 1.21 (3H, m, OCH_2CH_3) ppm. ^{13}C NMR ($CDCl_3$) 170.4, 170.2, 169.7 (3C, $COCH_3$), 161.7 (C-1), 134.0 (C-4), 127.3 (C-3), 95.5 (C-2), 71.7 (C-8), 70.8 (C-6), 68.6 (C-7), 64.8 (OCH_2CH_3), 62.5 (C-9), 52.9 ($COOCH_3$), 43.6 (C-5), 23.2 ($\underline{C}H_3CONH$), 20.9, 20.7, 20.5 (3C, $\underline{C}H_3COO$), 15.2 (OCH_2CH_3) ppm. MS (ESI positive) *m/z* 460.4 $[M+H]^+$, 482.4 $[M+Na]^+$. $C_{20}H_{29}NO_{11}$: calcd C, 52.28; H, 6.36; N, 3.05; found C, 51.71; H, 6.01; N, 2.91.

Preparation of methyl 5-acetamido-2,6-anhydro-2-*n*-propyl-3,4,5-trideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (64)

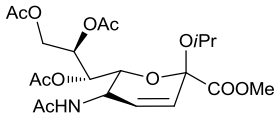


64

Starting from the oxazoline **16** with *n*PrOH (150 µL, 2.0 mmol) the compound **64** is obtained after 1 hours at 50 °C, as a white solid (83.3 mg, 88%). Compound **64** shows: m.p. 129-131 °C; $[\alpha]_D$ – 53.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 5.99 (1H, dd, $J_{3,5}$ = 2.4, $J_{3,4}$ = 10.2 Hz, 3-H), 5.90 (1H, dd, $J_{4,5}$ = 1.6, $J_{4,3}$ = 10.2 Hz, 4-H), 5.41-5.36 (2H, d, overlapping, N-H and 7-H), 5.30 (1H, ddd, $J_{8,9a}$ = 2.4, $J_{8,7}$ = $J_{8\text{-}9b}$ = 6.1 Hz, 8-H), 4.66-4.58 (2H, overlapping, 9a-H and 5-H), 4.23 (1H, dd, $J_{9b,8}$ = 6.1, $J_{9b,9a}$ = 12.6 Hz, 9b-H), 4.02 (1H, dd, $J_{6,7}$ = 2.3, $J_{6,5}$ = 10.3 Hz, 6-H), 3.82 (3H, s, $COOCH_3$), 3.67 (1H, m, $OCH_aCH_2CH_3$), 3.38 (1H, m, $OCH_bCH_2CH_3$), 2.10 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 1.97 (3H, s, CH_3CONH), 1.60 (2H, m, $OCH_2CH_2CH_3$), 0.92 (3H, m, $OCH_2CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$) 170.4, 170.2, 169.7 (3C, $COCH_3$), 161.7 (C-1), 134.0 (C-4), 127.3 (C-3), 95.5 (C-2), 71.7 (C-8), 70.8 (C-6), 70.7 (OCH_2CH_2), 68.6 (C-7), 64.8 (OCH_2CH_3), 62.5 (C-9), 52.9 ($COOCH_3$), 43.6 (C-5), 23.2 ($\underline{C}H_3CONH$), 22.7 ($OCH_2CH_2CH_3$), 20.9, 20.7, 20.5 (3C, $\underline{C}H_3COO$), 10.9

($OCH_2CH_2CH_3$) ppm. MS (ESI positive) *m/z* 474.4 $[M+H]^+$, 496.5 $[M+Na]^+$. $C_{21}H_{31}NO_{11}$: calcd C, 53.27; H, 6.60; N, 2.96; found C, 52.48; H, 6.09; N, 2.81.

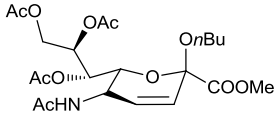
Preparation of methyl 5-acetamido-2,6-anhydro-2-*O*-*i*-propyl-3,4,5-trideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (66)



66

Starting from the oxazoline **16** with *i*PrOH (153 µL, 2.0 mmol) the compound **66** is obtained after 1 hours at 50 °C, as a white solid (64.4 mg, 68%). Compound **66** shows: m.p. 120-122 °C; $[\alpha]_D$ – 30.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.03 (1H, dd, $J_{3,5}$ = 2.4, $J_{3,4}$ = 10.0 Hz, 3-H), 5.90 (1H, dd, $J_{4,5}$ = 1.6, $J_{4,3}$ = 10.0 Hz, 4-H), 5.41-5.36 (2H, d, overlapping, N-H and 7-H), 5.31 (1H, ddd, $J_{8,9a}$ = 2.6, $J_{8,7}$ = $J_{8\text{-}9b}$ = 6.2 Hz, 8-H), 4.66-4.58 (2H, overlapping, 9a-H and 5-H), 4.23 (1H, dd, $J_{9b,8}$ = 6.2, $J_{9b,9a}$ = 12.6 Hz, 9b-H), 4.07 (1H, dd, $J_{6,7}$ = 2.3, $J_{6,5}$ = 10.3 Hz, 6-H), 3.83-3.71 (4H, overlapping, $COOCH_3$ and $OC\underline{H}(CH_3)_2$), 2.09 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 1.97 (3H, s, CH_3CONH), 1.20 (3H, d, $J_{CH,(CH3)a}$ = 6.1 Hz, $OCH(C\underline{H}_3)$a), 1.16 (3H, d, $J_{CH,(CH3)b}$= 6.1 Hz, $OCH(C\underline{H}_3)$b) ppm. ^{13}C NMR ($CDCl_3$) 170.5, 170.3, 169.9 (3C, $COCH_3$), 161.7 (C-1), 134.5 (C-4), 127.1 (C-3), 95.6 (C-2), 71.5 (C-8), 71.7 ($O\underline{C}H(CH_3)_2$), 70.8 (C-6), 68.6 (C-7), 64.8 (OCH_2CH_3), 62.5 (C-9), 52.9 ($COOCH_3$), 43.6 (C-5), 23.3 ($OCH(\underline{C}H_3)$a), 21.7 ($OCH(\underline{C}H_3)$b), 20.9, 20.7, 20.5 (3C, $\underline{C}H_3COO$) ppm. MS (ESI positive) *m/z* 474.5 $[M+H]^+$, 496.4 $[M+Na]^+$. $C_{21}H_{31}NO_{11}$: calcd C, 53.27; H, 6.60; N, 2.96; found C, 52.36; H, 6.11; N, 2.79.

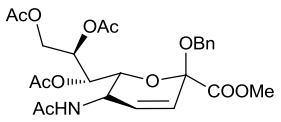
Preparation of methyl 5-acetamido-2,6-anhydro-2-*n*-butyl-3,4,5-trideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (65)



65

Starting from the oxazoline **16** with *n*BuOH (182 µL, 2.0 mmol) the compound **65** is obtained after 1 hours at 50 °C, as a white solid (81.9 mg, 84%). Compound **65** shows: m.p. 110-112°C; $[\alpha]_D$ – 36.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 5.91 (1H, dd, $J_{3,5}$ = 2.5, $J_{3,4}$ = 10.1 Hz, 3-H), 5.84 (1H, dd, $J_{4,5}$ = 1.8 , $J_{4,3}$ = 10.1 Hz, 4-H), 5.62 (1H, d, $J_{NH,5}$ = 9.7 Hz, N-H), 5.36 (1H, dd, $J_{7,6}$ = 2.6, $J_{7,8}$ = 4.2 Hz, 7-H), 5.23 (1H, ddd, $J_{8,9a}$ = 2.3, $J_{8,7}$ = 4.2, $J_{8\text{-}9b}$ = 7.0 Hz, 8-H), 4.70-4.61 (2H, overlapping, 9a-H and 5-H), 4.23 (1H, dd, $J_{9b,8}$ = 7.0, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 4.05 (1H, dd, $J_{6,7}$ = 2.6, $J_{6,5}$ = 10.2 Hz, 6-H), 3.77 (3H, s, $COOCH_3$), 3.54 (1H, m, $OCH_a(CH_2)_2H_3$), 3.30 (1H, m, $OCH_b(CH_2)_2CH_3$), 2.12 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.00 (3H, s, CH_3COO), 1.94 (3H, s, CH_3CONH), 1.56-1.46 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.38-1.29 (2H, m, $O(CH_2)_2CH_2CH_3$), 0.89 (3H, t, $OCH_2CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$) 170.5 (CH_3CONH), 170.4, 170.0, 169.8 (3C, $COCH_3$), 167.7 (C-1), 133.2 (C-4), 126.2 (C-3), 96.0 (C-2), 71.8 (C-8), 70.8 (C-6), 68.7 (C-7), 64.6 ($OCH_2(CH_2)_2CH_£$), 62.5 (C-9), 52.6 ($COOCH_3$), 43.0 (C-5), 31.8 ($OCH_2CH_2CH_2CH_3$), 23.2 (CH_3CONH), 20.9, 20.7 (3C, CH_3COO), 19.1 ($O(CH_2)_2CH_2CH_3$), 13.8 ($O(CH_2)_3CH_3$) ppm. MS (ESI positive) *m/z* 488.5 $[M+H]^+$, 510.5 $[M+Na]^+$. $C_{22}H_{33}NO_{11}$: calcd C, 54.20; H, 6.82; N, 2.87; found C, 53.07; H, 6.33 N, 2.54.

Preparation of methyl 5-acetamido-2,6-anhydro-2-benzyl-3,4,5-trideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (67)

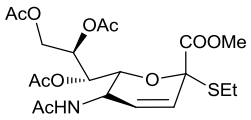


67

Starting from the oxazoline **16** with BnOH (207 µL, 2.0 mmol) the compound **67** is obtained after 1 hours at 50 °C, as a white solid (90.7 mg, 87%). Compound **67** shows: m.p. 109-111°C; $[\alpha]_D^{20}$ – 44.3 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.38-7.25 (4H, overlapping, Ph), 6.00 (1H, dd, $J_{3,5}$ = 2.5, $J_{3,4}$ = 10.1 Hz, 3-H), 5.90 (1H, dd, $J_{4,5}$ = 1.7, $J_{4,3}$ = 10.1 Hz, 4-H), 5.54 (1H, d, $J_{NH,5}$ = 9.6 Hz, N-H), 5.39 (1H, dd, $J_{7,6}$ = 2.4, $J_{7,8}$ = 4.9 Hz, 7-H), 5.33 (1H, ddd, $J_{8,9a}$ = 2.2, $J_{8,7}$ =

4.9, $J_{8\text{-}9b}$ = 6.8 Hz, 8-H), 4.71-4.60 (3H, overlapping, 9a-H, 5-H, CHaPh), 4.48 (1H, d, $J_{Hb,Ha}$ = 11.5 Hz, CHbPh), 4.23 (1H, dd, $J_{9b,8}$ = 6.8, $J_{9b,9a}$ = 12.5 Hz, 9b-H), 4.14 (1H, dd, $J_{6,7}$ = 2.4, $J_{6,5}$ = 10.3 Hz, 6-H), 3.74 (3H, s, $COOCH_3$), 2.15 (3H, s, CH_3COO), 2.02 (3H, s, CH_3COO), 1.95 (3H, s, CH_3COO), 1.92 (3H, s, CH_3CONH) ppm. ^{13}C NMR ($CDCl_3$) 170.5 ($CH_3\underline{C}ONH$), 170.5, 170.1, 169.9 (3C, $\underline{C}OCH_3$), 167.5 (C-1), 137.0-125.9 (6C, Ph), 133.4 (C-4), 127.8 (C-3), 96.1 (C-2), 71.2 (C-8), 70.7 (C-6), 68.5 (C-7), 66.5 ($\underline{C}H_2Ph$), 62.4 (C-9), 52.7 ($COOCH_3$), 43.1 (C-5), 23.2 ($\underline{C}H_3CONH$), 20.8, 20.7 (3C, $\underline{C}H_3COO$) ppm. MS (ESI positive) *m/z* 522.5 $[M+H]^+$, 544.5 $[M+Na]^+$. $C_{25}H_{31}NO_{11}$: calcd C, 57.58; H, 5.99; N, 2.69; found C, 57.00; H, 5.31; N, 2.46.

Preparation of methyl 5-acetamido-2,6-anhydro-2-ethylthio-2,3,4,5-tetradeoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (59)

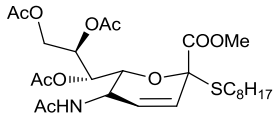


59

Starting from the oxazoline **16** with EtSH (144 µL, 2.0 mmol) the compound **59** is obtained after 1 hours at 50 °C, as a white solid (87.5 mg, 92%). Compound **59** shows: m.p. 147-149°C; $[\alpha]_D^{20}$ + 25.1 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.10 (1H, dd, $J_{3,5}$ = 2.2, $J_{3,4}$ = 10.1 Hz, 3-H), 5.74 (1H, dd, $J_{4,5}$ = 1.3 , $J_{4,3}$ = 10.1 Hz, 4-H), 5.59 (1H, d, $J_{NH,5}$ = 9.5 Hz, N-H), 5.38 (1H, dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 3.6 Hz, 7-H), 5.22 (1H, m, 8-H), 4.66-4.58 (2H, overlapping, 9a-H and 5-H), 4.33 (1H, dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.0 Hz, 6-H), 4.24 (1H, dd, $J_{9b,8}$ = 6.8, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 2.64-2.53 (2H, m, $CH_3C\underline{H_2}S$), 2.13 (3H, s, CH_3COO), 2.08 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.96 (3H, s, CH_3CONH), 1.16 (3H, t, $SCH_2C\underline{H_3}$) ppm. ^{13}C NMR ($CDCl_3$) 170.5 ($CH_3\underline{C}ONH$), 170.4, 170.2, 169.8 (3C, $\underline{C}OCH_3$), 167.3 (C-1), 130.7 (C-4), 126.2 (C-3), 85.7 (C-2), 71.4 (C-8), 70.7 (C-6), 68.5 (C-7), 62.5 (C-9), 52.8 ($COOCH_3$), 43.1 (C-5), 24.4 ($CH_3\underline{C}H_2S$), 23.3 ($\underline{C}H_3CONH$), 21.0, 20.7 (3C, $\underline{C}H_3COO$), 14.1 ($\underline{C}H_3CH_2S$) ppm. MS (ESI positive)

m/z 476.1 $[M+H]^+$, 498.5 $[M+Na]^+$. $C_{20}H_{29}NO_{10}S$: calcd C, 50.52; H, 6.15; N, 2.95; found C, 49.91; H, 6.03; N, 2.50

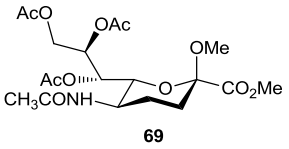
Preparation of methyl 5-acetamido-2,6-anhydro-2-*n*octylthio-2,3,4,5-tetradeoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-3-enonate (68)



68

Starting from the oxazoline **16** with $C_8H_{17}SH$ (144 µL, 2.0 mmol) the compound **68** is obtained after 1 hours at 50°C, as a white solid (66.0 mg, 59%). Compound **68** shows: m.p. 106-108°C; $[\alpha]_D$ + 28.9 (*c* 1 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.13 (1H, dd, $J_{3,5}$ = 2.5, $J_{3,4}$ = 10.1 Hz, 3-H), 5.74 (1H, dd, $J_{4,5}$ = 2.0, $J_{4,3}$ = 10.1 Hz, 4-H), 5.43-5.38 (2H, overlapping, N-H and 7-H), 5.20 (1H, ddd, $J_{8,9a}$ = 2.3, $J_{8,7}$= 4.2, $J_{8,9b}$ = 7.0, 8-H), 4.69-4.62 (2H, overlapping, 9a-H and 5-H), 4.35 (1H, dd, $J_{6,7}$ = 2.5, $J_{6,5}$ = 10.0 Hz, 6-H), 4.27 (1H, dd, $J_{9b,8}$ = 7.0, $J_{9b,9a}$ = 12.4 Hz, 9b-H), 3.81 (3H, s, $COOCH_3$), 2.62-2.51 (2H, m, $CH_3C\underline{H_2}S$), 2.14 (3H, s, CH_3COO), 2.09 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.97 (3H, s, CH_3CONH), 1.38-1.20 (12H, overlapping, $SCH_2(\underline{CH_2})_6CH_3$), 0.87 (3H, t, $S(CH_2)_7C\underline{H_3}$) ppm. ^{13}C NMR ($CDCl_3$) 170.5 ($CH_3\underline{C}ONH$), 170.4, 170.2, 169.7 (3C, $\underline{C}OCH_3$), 167.4 (C-1), 130.5 (C-4), 126.5 (C-3), 85.5 (C-2), 71.8 (C-8), 70.8 (C-6), 68.7 (C-7), 62.6 (C-9), 52.8 ($COOCH_3$), 43.0 (C-5), 31.8-22.6 (7C, $S(\underline{C}H_2)_7CH_3$), 23.3 ($\underline{C}H_3CONH$), 21.0, 20.7 (3C, $\underline{C}H_3COO$), 14.0 ($\underline{C}H_3CH_2S$) ppm. MS (ESI positive) *m/z* 560.7 $[M+H]^+$, 582.7 $[M+Na]^+$. $C_{20}H_{29}NO_{10}S$: calcd C, 55.80; H, 7.38; N, 2.50; found C, 54.76; H, 6.99; N, 2.21.

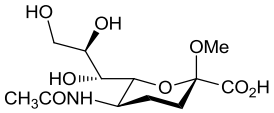
Preparation of methyl 5-acetamido-2-methyl-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (69)



69

Compound **62a** (90 mg, 0.2 mmol) is trated with Pd/C (9 mg, 10% w/w) and stirred in MeOH (10 mL) under H_2 atmosphere at 25 °C. After two hours, the catalyst is filtred, the solvent evaporated at reduced pressure and compound **69** obatained as whithe solid (88mg, 98%). Compound **69** shows: m.p. 142–144 °C; $[\alpha]_D$ + 69.3 (c 1 in $CHCl_3$); (Found: C, 50.53; H, 6.03; N, 2.94; Calcd for: $C_{19}H_{29}NO_{11}$ C, 51.00; H, 6.53; N, 3.13 %); ^{1}H NMR ($CDCl_3$) δ 5.65 (1H, d, $J_{NH,5}$= 9.8 Hz, N-H), 5.38 (1H, dd, $J_{7,8}$ = 5.9, $J_{7,6}$ = 2.4 Hz, 7-H), 5.18 (1H, ddd, $J_{8,9a}$ = 2.4, $J_{8,7}$ = 5.9, $J_{8,9b}$ = 7.8 Hz, 8-H), 4.81 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.4 Hz, 9a-H), 4.09 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 7.8 Hz, 9b-H), 3.97 (1H, m, 5-H), 3.81 (1H, dd, $J_{6,5}$ = 10.4, $J_{6,7}$ = 2.4 Hz, 6-H), 3.75 (3H, s, $COOCH_3$), 3.22 (3H, s, OCH_3), 2.10 (3H, s, CH_3COO), 2.02 (3H, s, CH_3COO), 2.01–1.99 (2H, overlapping, 3a-H, 3b-H), 1.98 (3H, s, CH_3COO), 1.87 (3H, s, CH_3CONH), 1.80-1.74 (2H, overlapping, 4aH, 4b-H); ^{13}C NMR ($CDCl_3$) δ 170.7, 170.5, 170.1, 169.6 (4C, 3XCH_3COO and $NHCOCH_3$), 168.6 (C-1), 97.9 (C-2), 72.7 (C-6), 71.2 (C-8), 68.9 (C-7), 62.5 (C-9), 52.4 (OCH_3), 51.0 ($COOCH_3$), 43.7 (C-5), 31.6 (C-3), 25.7 (C-4), 23.2 ($NHCOCH_3$), 20.9, 20.7 (3C, 3XCH_3COO); MS (ESI positive) m/z 448.4 $[M + H]^+$, 470.2 $[M + Na]^+$, 917.8 $[2M + Na]^+$.

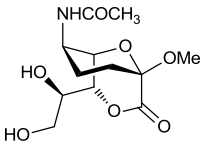
Preparation of 5-acetamido-2-methyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidic acid (70)



70

The protected compound **69** (80 mg, 0.18 mmol), dissolved in aqueous methanol (1.0 mL, 1:2 v/v) saturated with K_2CO_4, is stirred for 12 h at 23 °C. Then the solution is treated with a acidic resin [DOWEX 50WX8 (H^+)] and stirred for 15 min. The solution is filtered and the solvent removed under reduced pressure to afford the free compound **70** (50 mg, 90%), as a white solid: m.p. 142–144 °C; $[\alpha]_D$ + 71.0 (c 1 in MeOH); (Found: C, 47.39; H, 7.21; N, 4.99; Calcd for: $C_{12}H_{21}NO_8$ C, 46.90; H, 6.89; N, 4.55 %); ^{1}H NMR (MeOD) δ 4.01 (1H, m, 8-H), 3.90-3.78 (3H, overlapping, 9a-H, 9b-H, H-6), 3.68 (1H, m, 5-H), 3.57 (1H, m, 7-H), 3.33 (3H, s, OCH_3), 2.05-1.91 (6H, overlapping, 3a-H, 3b-H, $NHCOCH_3$, 4a-H), 1.78 (1H, m, 4b-H); ^{13}C NMR (MeOD) δ 173.8 ($NHCOCH_3$), 173.4 (C-1), 99.5 (C-2), 73.4 (C-6), 71.2 (C-8), 70.1 (C-7), 65.2 (C-9), 51.6 (OCH_3), 45.8 (C-5), 33.1 (C-3), 25.9 (C-4), 22.7 ($NHCOCH_3$); MS (ESI negative) m/z 307.1 $[M - H]^-$.

Preparation of 2-methyl-5-*N*-acetyl-4-deoxy-β-neuraminic acid 1,7 lactone (71)

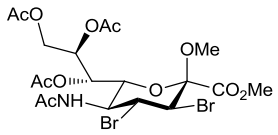


71

CbzCl (0.2 mL, 1.4 mmol), dissolved in THF (0.75 mL) is added dropwise to a stirred solution of anhydrous THF (1.3 mL) containing trietylamine (0.3 mL, 17 mmol), at 0 °C. Then, compound **70** (50 mg, 0.17 mmol) dissolved in DMF (1.5 mL) is added and the mixture is stirred at 25 °C, for 24 h. At this time, MeOH (0.5 mL) is added and stirring continued for 1 h[103]. After evaporation of the

solvent under high vacuum (0.1 mmHg), a crude residue is obtained and, after purification by flash chromatography (eluting with AcOEt/MeOH, 9:1, v/v), the pure lactone **71** is afforded (35.8 mg, 73%), as glass: $[\alpha]_D$ = + 25.0, (*c* 1, CH_3OH); ^1H NMR (CD_3OD) δ 4.61 (1H, br s, H-6), 4.38 (1H, d, $J_{7,8}$ = 8.1 Hz, H-7), 4.01 (1H, br s, H-5), 3.82-3.77 (2H, overlapping, H-8 and H-9a), 3.72 (1H, $J_{9b,9a}$ = 11.7, $J_{9b,8}$ = 5.6 Hz; H-9b), 3.39 (1H, s, $COOCH_3$), 2.06-1.92 (6H, overlapping, CH_3CONH, H-3a, H-4a and H-4b), 1.83-1.77 (1H, m,H-3b), 2.01 (3H, s, CH_3CONH); ^{13}C NMR (CD_3OD) δ 173.0 ($CH_3\underline{C}ONH$), 168.8 (C-1), 99.0, (C-2), 80.9 (C-7), 73.3 (C-6), 72.8 (C-8), 63.5 (C-9), 52.4 (1C, OCH_3), 47.7 (C-5), 30.0 (C-3), 24.4 (C-4), 22.5 ($\underline{C}H_3CONH$); MS (ESI negative) *m/z* 304.1 $[M-H]^-$. $C_{12}H_{19}NO_8$: calcd C, 47.21; H, 6.27; N, 4.59. Found: C, 47.16; H, 6.32; N, 4.49.

Preparation of methyl 5-acetamido-2-methyl-3,4-dibromo-7,8,9-tri-*O*-acetyl-3,4,5- trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (72)

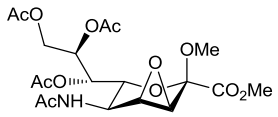


72

Compound **62a** (90 mg, 0.2 mmol) is stirred with Br_2 (16µL, 0.3 mmol) in DCM (2 mL) under Ar atmosphere for 30 minutes at 25 °C. After evaporation of the solvent under reduced pressure, a crude residue is obtained and, after purification by flash chromatography (eluting with AcOEt/Hexane, 7:3, v/v), the pure compound **72** is afforded (98 mg, 81%): m.p. 106–109 °C; $[\alpha]_D$ + 65.3 (c 1 in $CHCl_3$); (Found: C, 36.89; H, 4.09; N, 2.10; Calcd for: $C_{19}H_{27}Br_2NO_{11}$ C, 37.71; H, 4.50; N, 2.31 %); ^1H NMR ($CDCl_3$) δ 5.65-5.53 (2H, overlapping, 7-H and N-H), 5.18 (1H, ddd, $J_{8,9a}$ = 2.2, $J_{8,7}$ = 5.9, $J_{8,9b}$ = 8.1 Hz, 8-H), 4.97 (1H, dd, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, 4-H), 4.44 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.2 Hz, 9a-H), 4.34-4.23 (3H, overlapping, 9b-H, 5-H, 6-H), 4.03 (1H, $J_{3,4}$ = 9.6 Hz, 3-H), 3.85 (3H, s, $COOCH_3$), 3.46 (3H, s, OCH_3), 2.16 (3H, s, CH_3COO), 2.10 (3H, s, CH_3COO), 2.09 (3H, s, CH_3COO), 2.04 (3H, s, CH_3CONH); ^{13}C NMR ($CDCl_3$) δ 170.7,

170.5, 170.1, 169.6 (4C, 3XCH_3COO and $NHCOCH_3$), 166.1 (C-1), 100.2 (C-2), 80.0 (C-3), 71.2 (C-6), 70.7 (C-8), 66.1 (C-4), 68.8 (C-7), 61.5 (C-9), 52.8(OCH_3), 51.9 ($COOCH_3$), 49.0 (C-5), 23.3 ($NHCOCH_3$), 20.9, 20.7, 20.6 (3C, 3XCH_3COO); MS (ESI positive) m/z 606.2 $[M + H]^+$, 628.2 $[M + Na]^+$, 1233.5 $[2M + Na]^+$.

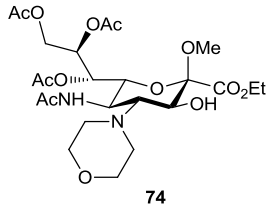
Preparation of methyl 5-acetamido-2-methyl-3,4-epoxide-7,8,9-tri-*O*-acetyl-3,4,5- trideoxy-D-*glycero*-β-D-*talo*-non-2-ulopyranosidonate (73)



73

Compound **62a** (90 mg, 0.2 mmol) is stirred with *m*CPBA (69 mg, 0.4 mmol) in MeCN (2 mL) for 8 hours at 80 °C. After evaporation of the solvent under reduced pressure, a crude residue is obtained and, after purification by flash chromatography (eluting with AcOEt/Hexane, 9:1, v/v), the pure compound **73** is afforded (73 mg, 79%): m.p. 153–155 °C; $[\alpha]_D$ + 26.6 (c 1 in $CHCl_3$); (Found: C, 48.79; H, 5.57; N, 2.90; Calc. for: $C_{19}H_{27}NO_{12}$ cald C, 49.46; H, 5.90; N, 3.04;%); ^1H NMR ($CDCl_3$) δ 5.74 (1H, d, $J_{NH,5}$ = 10.0 Hz, N–H), 5.31 (1H, dd, $J_{7,8}$ = 6.4, $J_{7,6}$ = 2.4 Hz, 7-H), 5.18 (1H, ddd, $J_{8,9b}$ = $J_{8,7}$ = 6.4, $J_{8,9a}$ = 2.4 Hz, 8-H), 4.57 (1H, ddd, $J_{5,NH}$ = $J_{5,6}$ = 10.0, $J_{5,4}$ = 1.4 Hz, 5-H), 4.51 (1H, dd, $J_{9a,9b}$ = 12.5, $J_{9a,8}$ = 2.4 Hz, 9a-H), 4.13 (1H, dd, $J_{9b,9a}$ = 12.5, $J_{9b,8}$ = 6.4 Hz, 9b-H), 4.00 (1H, dd, $J_{6,5}$ = 10.0, $J_{6,7}$ = 2.4 Hz, 6-H), 3.85 (3H, s, $COOCH_3$), 3.76 (1H, d, $J_{3,4}$ = 4.1 Hz, 3-H), 3.41 (1H, dd, $J_{4,3}$ = 4.1, $J_{4,5}$ = 1.4 Hz, 4-H), 3.34 (3H, s, OCH_3), 2.11 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.00 (3H, s, CH_3COO), 1.97 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.5, 170.3, 170.1, 169.7 (4C, 3XCH_3COO and $NHCOCH_3$), 166.8 (C-1), 95.3 (C-2), 71.0 (C-6), 67.8 (C-8), 67.4 (C-7), 62.1 (C-9), 56.0 (C-3), 54.2 (C-4), 53.0 ($COOCH_3$), 52.0 (OCH_3), 44.0 (C-5), 23.1 ($NHCOCH_3$), 20.9, 20.6 (3C, 3XCH_3COO); MS (ESI positive) m/z 462.4 $[M + H]^+$, 484.4 $[M + Na]^+$, 945.7 $[2M + Na]^+$.

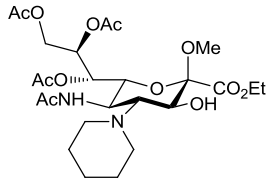
Preparation of methyl 5-acetamido-2-etyl-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-4,5- dideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (74)



74

Compound **73** (93 mg, 0.2 mmol) is stirred with morpholine (173 µL, 2.0 mmol) in EtOH (2 mL) for 12 hours at 80 °C. After evaporation of the solvent under reduced pressure, a crude residue is obtained and, after purification by flash chromatography (eluting with AcOEt/Hexane, 95:0.5 v/v), the pure compound **74** is afforded (91 mg, 83%): m.p. 133–135 °C; $[\alpha]_D$ + 38.6 (c 1 in $CHCl_3$); (Found: C, 49.51; H, 5.78; N, 4.33; Calcd. for: $C_{24}H_{38}N_2O_{13}$ C, 51.24; H, 6.81; N, 4.98 %); ^{1}H NMR ($CDCl_3$) δ 5.46 (1H, d, $J_{NH,5}$ = 9.8 Hz, N-H), 5.36 (1H, dd, $J_{7,8}$ = 6.0, $J_{7,6}$ = 2.3 Hz, 7-H), 5.15 (1H, ddd, $J_{8,9b}$ = 2.4, $J_{8,7}$ = 6.0, $J_{8,9a}$ = 7.8 Hz, 8-H), 4.71 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.4 Hz, 9a-H), 4.35-4.23 (2H, overlapping, 5-H and 9b-H), 4.10–4.04 (2H, $COOC\underline{H_2}CH_3$), 3.88 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.3 Hz, 6-H), 3.76 (1H, dd, $J_{4,5}$ = $J_{4,3}$ = 10.2 Hz, 4-H), 3.68 (1H, dd, $J_{3,4}$ = 10.2, $J_{3,OH}$ = 4.7 Hz, 3-H), 3.64 (1H, d,$J_{OH,3}$ = 4.7 Hz, OH), 3.60-3.53 (4H, overlapping, $N(C\underline{H_2}CH_2)_2O$), 3.40 (3H, s, OCH_3), 2.90-2.84 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.70–2.64 (2H, overlapping, N $(CH_2CH_2)_2O$), 2.12 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.00 (3H, s, CH_3COO), 1.92 (3H, s, CH_3CONH), 1.32 (3H, t, $OCH_2C\underline{H_3}$); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.5, 170.3, 170.0 (4C, 3 X CH_3COO and $NHCOCH_3$), 167.1 (C-1), 99.1 (C-2), 72.5 (C-6), 72.2 (C-8), 70.6 (C-7), 68.7 ($O\underline{C}H_2CH_3$), 67.9 (2C, N $(CH_2CH_2)_2O$), 62.4 (C-9), 62.2 (C-4), 51.6 (OCH_3), 49.8 (2C, $N(CH_2CH_2)_2O$), 46.0 (C-3), 40.6 (C-5), 23.3 ($NHCOCH_3$), 21.0, 20.8, 20.7 (3C, 3XCH_3COO), 15.1, $CH_2\underline{C}H_3$); MS (ESI positive) m/z 563.3 $[M + H]^+$, 585.3, $[M + Na]^+$, 1146.4 $[2M + Na]^+$.

Preparation of methyl 5-acetamido-2-ethyl-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-4,5- dideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (75)



75

Compound **73** (93 mg, 0.2 mmol) is stirred with piperidine (198 µL, 2.0 mmol) in EtOH (2 mL) for 12 hours at 80 °C. After evaporation of the solvent under reduced pressure, a crude residue is obtained and, after purification by flash chromatography (eluting with AcOEt), the pure compound **75** is afforded (87.4 mg, 80%): m.p. 133–135 °C; $[\alpha]_D$ + 37.1 (c 1 in $CHCl_3$); (Found: C, 52.99; H, 6.82; N, 4.79; Calc. for: $C_{25}H_{40}N_2O_{12}$ C, 53.56; H, 7.19; N, 5.00 %); ^{1}H NMR ($CDCl_3$) δ 5.33 (1H, dd, $J_{7,8}$ = 6.0, $J_{7,6}$ = 2.4 Hz, 7-H), 5.21 (1H, ddd, $J_{8,9b}$ = 2.3, $J_{8,7}$ = 6.0, $J_{8,9a}$ = 7.4 Hz, 8-H), 5.16 (1H, d, $J_{NH,5}$ = 9.9 Hz, N-H), 4.73 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.3 Hz, 9a-H), 4.35-4.23 (2H, overlapping, 5-H and 9b-H), 4.10–4.04 (2H, COOC<u>H</u>$_2$CH$_3$), 3.88 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.3 Hz, 6-H), 3.76 (1H, dd, $J_{4,5}$ = $J_{4,3}$ = 10.2 Hz, 4-H), 3.68 (1H, dd, $J_{3,4}$ = 10.2, $J_{3,OH}$ = 4.7 Hz, 3-H), 3.41 (3H, s, OCH_3), 2.98 (1H, d,$J_{OH,3}$ = 4.7 Hz, OH), 2.75-2.60 (4H, overlapping, N(C<u>H</u>$_2$CH$_2$)$_2$CH$_2$), 2.12 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.00 (3H, s, CH_3COO), 1.92 (3H, s, CH_3CONH), 1.46-1.38 (6H, N(CH_2CH_2)$_2$$CH_2$), 1.32 (3H, t, OCH_2C<u>H</u>$_3$) ; ^{13}C NMR ($CDCl_3$) δ 170.7, 170.4, 170.2, 170.0 (4C, 3 X CH_3COO and $NHCOCH_3$), 167.3 (C-1), 99.0 (C-2), 72.4 (C-6), 72.3 (C-8), 70.6 (C-7), 68.7 (O<u>C</u>H$_2$CH$_3$), , 62.6 (C-9), 62.2 (C-4), 51.5 (OCH_3), 50.5 (2C, N(CH_2CH_2)$_2$$CH_2$), 46.2 (C-3), 40.9 (C-5), 27.2 (2C, N(CH_2CH_2)$_2$$CH_2$), 24.7 (1C, N($CH_2CH_2$)$_2$$CH_2$), 23.2 ($NHCOCH_3$), 20.9, 20.8, 20.7 (3C, 3XCH_3COO), 15.1, CH_2<u>C</u>H$_3$); MS (ESI positive) m/z 561.6 $[M + H]^+$, 583.5, $[M + Na]^+$, 1144.1 $[2M + Na]^+$.

5.3.6 Biological assays

Inhibition of *Vibrio cholerae* neuraminidase activity

Inhibition of sialidase activity is performed essentially according to essentially according to Venerando et al.[109], but using the fluorescent artificial substrate 4-methylumbelliferyl *N*-acetylneuraminic acid (4-MU-Neu5Ac). Briefly, the incubation mixture (final volume of 100 µL) contained 0.1–0.5 mU of *Vibrio cholerae* neuraminidase, various amounts of inhibitors (0-500 mM), 0.3 mM 4-MU-Neu5Ac, 600 µg of bovine serum albumin (BSA), 25 mM of Tris/HCl buffer (pH 6.9). After incubation at 37 °C for 5–10 min, the reactions are stopped by the addition of 1.5 mL of 0.2 M glycine buffered with NaOH at pH 10.8, and neuraminidase activity is determined by spectrofluorometric measurement of the 4-methylumbelliferone released (λ excitation 365 nm, λ emission 448 nm). One unit of neuraminidase is defined as the amount of enzyme releasing 1 µmol of *N*-acetylneuraminic acid min^{-1} at 37 °C. IC_{50} values are obtained by linear regression (using Microsoft Excel) of different concentrations of inhibitors from 0 to 500 mM (five concentrations of each inhibitor are used to determine an IC_{50}) with a fixed concentration (0.3 mM) of 4-MU-Neu5Ac. Typical concentration–response plots are obtained from the average values of triplicate assay results.

Inhibition of *Clostridium perfringens* neuraminidase activity

Sialidase activity inhibition is performed, as the precedent one, using 4-MU-Neu5Ac as artifial substrate. Briefly, the incubation mixture (final volume of 100 µL) contained 0.1–0.5 mU of *Clostridium perfringens* neuraminidase, various amounts of inhibitors (0-500 mM), 0.3 mM 4-MU-Neu5Ac, 600 µg of bovine serum albumin (BSA), 2.5 µmol of CH_3COOH/CH_3COONa buffer (pH 5.3), in a final volume of 100 µL. After incubation at 37 °C for 10 min, the reactions are stopped by the addition of 1.5 mL of 0.2 M glycine buffered with NaOH at pH 10.8, and neuraminidase activity is determined by spectrofluorometric

measurement of the 4-methylumbelliferone released (λ excitation 365 nm, λ emission 448 nm). One unit of neuraminidase is defined as the amount of enzyme releasing 1 μmol of *N*-acetylneuraminic acid min^{-1} at 37 °C. IC_{50} values are obtained by linear regression (using Microsoft Excel) of different concentrations of inhibitors from 0 to 500 mM (five concentrations of each inhibitor are used to determine an IC_{50}) with a fixed concentration (0.3 mM) of 4-MU-Neu5Ac. Typical concentration–response plots are obtained from the average values of triplicate assay results.

Inhibition of Newcastle disease virus (NDV) neuraminidase activity

Neuraminidase activity inhibition assay is performed, as the precedent one, using using 4-MU-Neu5Ac as artificial substrate. Briefly, the incubation mixture (final volume of 100 μL) contained 0.1–0.5 mU of NDV "clone 30", various amounts of inhibitors (0-500mM), 0.3 mM 4-MU-Neu5Ac, 600 μg of bovine serum albumin (BSA), 200 mM sodium-citrate/phosphate buffer pH 6.8. After incubation at 37 °C for 10 min, the reactions are stopped by the addition of 1.5 mL of 0.2 M glycine buffered with NaOH at pH 10.8, and neuraminidase activity is determined by spectrofluorometric measurement of the 4-methylumbelliferone released (λ excitation 365 nm, λ emission 448 nm). One unit of neuraminidase is defined as the amount of enzyme releasing 1 μmol of *N*-acetylneuraminic acid min^{-1} at 37 °C. IC_{50} values are obtained by linear regression (using Microsoft Excel) of different concentrations of inhibitors from 0 to 500 mM (five concentrations of each inhibitor are used to determine an IC_{50}) with a fixed concentration (0.3 mM) of 4-MU-Neu5Ac. Typical concentration–response plots are obtained from the average values of triplicate assay results.

Inhibition of Neu3 sialidase activity[110]

In order to study sialidase inhibitor effects on mammalian plasma membrane-associated Neu3 sialidase we employ HeLa cell stable transfected with murine Neu3 gene (MmNEU3). MmNeu3 expressing HeLa cells are cultured in Dulbecco's Modified Eagle Medium (DMEM) with high glucose supplemented with 10% (v/v) fetal bovine serum (FBS) and 4mM glutamine, and are maintained at 37°C and 5% CO_2 in a humidified incubator. Cells are harvested by scraping in phosphate-buffered saline (PBS) at pH 7.4, centrifugated (250 x *g* for 10 minutes at 4°C), and the cellular pellet is resuspended in PBS buffer at pH 7.4 containing 1 mM EDTA, and a protease inhibitor mixture (10 µg/mL Aprotinin, 10 µg/mL Leupeptin, 10 µg/mL Pepstatin). Crude cell extracts are prepared by sonication and centrifugation at 800 x *g* for 10 minutes at 4°C to eliminate cell nuclei and unbroken cells. Successively, crude cell extracts are centrifugated at 200000 x *g* for 20 minutes at 4°C on TL100 ultracentrifuge (Beckman), to achieve the separation between the cytosolic and particulate fraction (containing cell membranes). Membrane fraction is resuspended in PBS buffer at pH 7.4, 1 mM containing EDTA, and a protease inhibitor mixture (10 µg/mL Aprotinin, 10 µg/mL Leupeptin, 10 µg/mL Pepstatin), and homogenated by a dounce homogenizer.
The protein concentration is determinated using the Bradford method, and aliquot of the particulate fraction are used for sialidase activity.

Protein quantification according to the Bradford method

The Bradford assay, a colorimetric protein assay, is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 from the reddish/brown form of the dye (absorbance maximum at 465nm) to the blue form of the dye (absorbance maximum at 610nm). In the acidic environment of the reagent protein binds to the coomassie dye and the binding stabilizes the blue form of the Coomassie dye. Thus the amount of the complex present in solution is a measure of the protein concentration, and can be estimated by use of an

absorbance reading. The coomassie dye-protein has a maximum in the absorption spectrum at 595 nm. By this method it is possible to detect protein amounts from 0.2 to 20 µg. Total protein concentration is calculated comparing sample absorbance to a standard curve generated by the reaction of known amounts of a standard protein, usually BSA.

Enzymatic assay

The sialidase activity of Neu3 is determined by measuring the fluorescence of 4-methylumbelliferone released by the hydrolysis of 0.3 mM 4-MU-Neu5Ac. Briefly, assays are performed in triplicate with 30 µg of particulate proteins, different concentrations of inhibitors (0-500 mM), 0.3 mM 4-MU-Neu5Ac, 600 µg of BSA, 12.5 mM sodium-citrate/phosphate buffer pH 3.8, in a final volume of 100 µl. After incubation at 37 °C for 30 min, the reactions are stopped by the addition of 1.5 mL of 0.2 M glycine pH 10.8, and fluorescence is measured with a spectrofluorometer (Jasco) (λ excitation 365 nm, λ emission 448 nm). One unit of sialidase activity is defined as the amount of enzyme releasing 1 µmol of *N*-acetylneuraminic acid min^{-1} at 37 °C. IC_{50} values are obtained by linear regression (using Microsoft Excel) of different concentrations of inhibitors from 0 to 500 mM (five concentrations of each inhibitor are used to determine an IC_{50}) with a fixed concentration (0.3 mM) of 4-MU-Neu5Ac. Typical concentration–response plots are obtained from the average values of triplicate assay results.

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