

Wayne State University Dissertations

January 2018

Stereoselective Synthesis Of Legionaminic Acid And Pseudaminic Acid Glycosides

Bibek Dhakal

Wayne State University, fi1189@wayne.edu

Follow this and additional works at: https://digitalcommons.wayne.edu/oa_dissertations

 Part of the [Organic Chemistry Commons](#)

Recommended Citation

Dhakal, Bibek, "Stereoselective Synthesis Of Legionaminic Acid And Pseudaminic Acid Glycosides" (2018). *Wayne State University Dissertations*. 2155.

https://digitalcommons.wayne.edu/oa_dissertations/2155

This Open Access Dissertation is brought to you for free and open access by DigitalCommons@WayneState. It has been accepted for inclusion in Wayne State University Dissertations by an authorized administrator of DigitalCommons@WayneState.

**STEREOSELECTIVE SYNTHESIS OF LEGIONAMINIC ACID AND
PSEUDAMINIC ACID GLYCOSIDES**

by

BIBEK DHAKAL

DISSERTATION

Submitted to the Graduate School of

Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2019

MAJOR: CHEMISTRY (Organic)

Approved By:

Advisor

Date

DEDICATION

This dissertation is dedicated to the five most important people in my life for their unflinching support and motivation- my parents, my brother, my wife, and especially my daughter Bianca.

ACKNOWLEDGEMENTS

First of all, I would like to express my utmost gratitude to my supervisor and mentor, Prof. David Crich for his infallible support, guidance, inspiration and encouragement during the entire length of my graduate studies, without which this thesis wouldn't have even started let alone completed. His depth of knowledge and the enthusiasm toward chemistry kept me going and achieve the targets. Particularly, his patience toward my failures and his belief in me and my unforeseen abilities will always be appreciated. Sincere thanks to all of my dissertation committee members, Prof. Jennifer L. Stockdill, Prof. Long Luo, and Prof. Xuefei Huang for the valuable time spent in reading, evaluating and suggesting my dissertation work. I would specially like to thank Prof. Luo for accepting to become my committee member in such a short notice at the last moment.

I would also like to thank past and present members of the Crich laboratory for providing amenable working environment in the laboratory. Particularly, I am thankful to Dr. Buda, Dr. Matsushita, Dr. Dharuman and Dr. Kato for their valuable suggestions and guidance during my initial days in the laboratory. Special words of gratitude to Dr. Vikram for the discussion sessions helping me move past the roadblocks encountered while running the experiments. I also thank Dr. Popik, Dr. Appi, Dr. Amr, Dr. Parasuraman, Dr. Peng, Dr. Girish and Sandeep for the courtesy shown toward me both on and off the laboratory. Also, sincere thanks to all the helpful staff members in the department of chemistry.

I am especially thankful to my wife Kanti Laxmi Dahal for her support at home and for her role in playing a bridging role between me and my family and relatives back

in my home country, Nepal. Not only the spiritual support, her adept culinary skills also provided me the vital physical support all along my graduate studies. Last but not the least, I am always indebted to my father and mother for instilling the good manners and cultures in me, which is why I am who I am.

TABLE OF CONTENTS

Dedication	ii
Acknowledgements	iii
List of Tables	x
List of Figures	xi
List of Schemes	xii
List of Abbreviations	xvi
CHAPTER 1. INTRODUCTION	1
1.1 Sialic acids	1
1.2 Biological roles of sialic acids	1
1.3 Legionaminic acid and its biological roles	5
1.4 Pseudaminic acid and its biological roles	8
1.5 Structure and linkage diversity in legionaminic and pseudaminic acid	11
1.6 Biosynthesis of legionaminic acid and pseudaminic acid	14
1.7 Synthesis of sialic acid glycoconjugates	17
1.7.1 Sialylation assisted by auxiliary groups	18
1.7.2 Conversion of the acetamide at C-5 to more electron withdrawing groups	20
1.7.3 Use of cyclic protecting groups	20
1.8 Synthesis of legionaminic acid and its glycosides	21
1.8.1 Tsvetkov's synthesis of Leg	21
1.8.2 Seeberger's synthesis of Leg and a glycoside	22
1.8.3 Whitfield's chemoenzymatic synthesis of Leg	23
1.8.4 Chen's chemoenzymatic synthesis of Leg and its glycosides	25

1.9 Synthesis of pseudaminic acid and its glycosides	27
1.9.1 Tsvetkov's synthesis of Pse	27
1.9.2 Ito's synthesis of Pse and its glycoside	28
1.9.3 Kiefel's synthesis of Pse	28
1.9.4 Li's synthesis of Pse and its glycosides	29
1.9.5 Chou's chemoenzymatic synthesis of Pse	31
1.9.6 Schoenhofen's chemoenzymatic synthesis of Pse	32
1.10 Analysis of expected difficulties arising from different configurations	32
1.10.1 Inversion of configuration at C-5 via oxidative deamination	33
1.10.2 Inversion of configuration at C-8 and C-7	34
1.10.3 Deoxygenation at C-9	35
1.10.4 Effect of C-5 stereochemistry on the glycosylation selectivity	35
1.11 Goals of this thesis	36
CHAPTER 2. STEREOSELECTIVE SYNTHESIS OF THE EQUATORIAL GLYCOSIDES OF LEGIONAMINIC ACID	38
2.1 Background	38
2.2 Results and discussion	39
2.2.1 Retrosynthetic analysis of the synthesis of a legionaminic acid donor	39
2.2.2 Synthesis of the 5- <i>N</i> -Boc protected thioadamantanyl sialoside 162	40
2.2.3 Selective functionalization of the 9-hydroxy group	41
2.2.4 Synthesis of a 9-iodo dibenzoylated sialoside	43
2.2.5 Deiodination and synthesis of 5-azido sialoside	43
2.2.6 Selective formation of a 7,8-di- <i>O</i> -benzoate	44
2.2.7 Inversion of configuration of the 7-hydroxy group	45

2.2.8 Synthesis of a 5,7-diazido Leg donor	46
2.2.9 Synthesis of a sialic acid acceptor	47
2.2.10 Glycosylation study with the Leg donor	48
2.2.11 Deprotection of regioisomeric equatorial-(2→6) galactosyl disaccharide	50
2.2.12 Deprotection of regioisomeric equatorial-(2→3) galactosyl disaccharide	51
2.2.13 Influence of configuration of C7 on side-chain conformation	52
2.2.14 Assignment of configuration for coupled sialosides	53
2.3 Conclusion	55
CHAPTER 3. STEREOSELECTIVE SYNTHESIS OF EQUATORIAL 5-EPI-SIALOSIDES RELATED TO THE PSEUDAMINIC ACID GLYCOSIDES	57
3.1 Background	57
3.2 Results and Discussion	58
3.2.1 Zbiral oxidative deamination of NeuAc thioglycoside with levulinic acid as nucleophile	58
3.2.2 Synthesis of a 5- <i>epi</i> -azido donor	62
3.2.3 Oxidative deamination of epimeric thioglycoside using levulinic acid	63
3.2.4 Oxidative deamination of a NeuAc thioglycoside using triflic acid as nucleophile	63
3.2.5 Glycosylation study with the 5- <i>epi</i> -azido donor 206	65
3.2.6 Use of 5-azido as protecting group in sialyl donors	66
3.2.7 Synthesis of a 5-azido NeuAc donor	71
3.2.8 Glycosylation study with the 5-azido NeuAc donor 288	72
3.2.9 Synthesis of the 5-azido equatorial thioglycoside donor	73
3.2.10 Stereoselective glycosylation with the equatorial thioglycoside donor 296	74
3.2.11 Deprotection of 5- <i>epi</i> -azido glycosides	75

3.2.12 Deprotection of 5-azido glycosides	76
3.2.13 Leaving group dependent stereoselectivity at the 5-position of NeuAc	77
3.2.14 Influence of the C5 configuration on side-chain conformation and glycosylation selectivity	79
3.3 Conclusion	81
CHAPTER 4. STEREOSELECTIVE SYNTHESIS OF PSEUDAMINIC ACID AND ITS EQUATORIAL GLYCOSIDES	83
4.1 Background	83
4.2 Results and Discussion	87
4.2.1 Retrosynthesis	87
4.2.2 Attempted synthesis of a Pse donor	88
4.2.2.1 Synthesis of a 7- <i>O</i> -TBDMS thioglycoside	88
4.2.2.2 Selective functionalization of 9-hydroxy group	89
4.2.2.3 Inversion of configuration of a 8-hydroxy group	89
4.2.2.4 Synthesis of a 9-deoxy sialoside	90
4.2.2.5 Attempted late stage oxidative deamination with levulinic acid	90
4.2.3 Synthesis of a Pse donor	92
4.2.3.1 Synthesis of a 7- <i>O</i> -Naphthyl thioadamantanyl sialoside	92
4.2.3.2 Oxidative deamination with levulinic acid as nucleophile	92
4.2.3.3 Selective functionalization of the 9-hydroxy group	93
4.2.3.4 Inversion of configuration of the 8-hydroxy group	94
4.2.3.5 Synthesis of a 9-deoxy sialoside	95
4.2.3.6 Deprotection of the 7-naphthyl and 5-levulinoyl groups	95
4.2.3.7 Synthesis of the 5,7-diazido Pse donor 311	96

4.2.3.8 Glycosylation study with the Pse donor	97
4.2.3.9 Analysis of side chain conformation and glycosylation selectivity	99
4.2.3.10 Synthesis of the final Pse5Ac7Ac	100
4.2.3.11 Regioselective reduction of azide in the deprotection of 345	101
4.2.3.12 Alternate regioselective reduction of azide in the deprotection of 345	103
4.2.3 Conclusion	103
CHAPTER 5. CONCLUSION	105
CHAPTER 6. EXPERIMENTAL SECTION	107
References	184
Abstract	202
Autobiographical Statement	205

LIST OF TABLES

Table 1: Structure of legionaminic acids found in different pathogenic bacteria	8
Table 2: Structures of pseudaminic acids found in different pathogenic bacteria	10
Table 3: Conditions attempted by the Kiefel group to form 164	42
Table 4: Glycosylation reactions of various acceptors with 181	49
Table 5: Key spectral parameters and side chain conformations of 174 , 179 and 181	53
Table 6: Glycosylation reactions with D-glycero-D-gulo donor 206	66
Table 7: Glycosylation reactions with D-glycero-D-galacto donor 288	73
Table 8: Deprotection of the 5-epi azido glycosides 232 and 233	76
Table 9: Deprotection of the 5-azido glycosides 204 and 290	77
Table 10: Glycosylation reactions of various acceptors with the Pse donor 311	99

LIST OF FIGURES

Figure 1: Fischer projections and cyclic structures of legionaminic acid, pseudaminic acid and <i>N</i> -acetylneuraminic acid	1
Figure 2: Naturally occurring sialoside linkages	3
Figure 3: Naturally occurring bacterial nonulosonate (NulO) sugars	6
Figure 4: Structures of polylegionaminic acid and the <i>O</i> -specific lipopolysaccharide from <i>P. aeruginosa</i> O10	12
Figure 5: Structure of a glycan from HRPV-1 containing legionaminic acid	12
Figure 6: Structure of Teichulosonic acid fragment in <i>Kribella sp</i> containing pseudaminic acid	13
Figure 7: Structure <i>P. aeruginosa</i> 1244 pilin containing pseudaminic acid	13
Figure 8: Sialic acid donors with 4 <i>O</i> ,5 <i>N</i> -oxazolidinone ring	21
Figure 9: Dihedral angles of equatorial and axial sialosides	54
Figure 10: Example of $^3J_{C-H}$ analysis using the SFORD experiment	55
Figure 11: O4,N5-oxazolidinone sialic acid 66 and its unnatural C-7 epimer 205	57
Figure 12: Planned 5- <i>epi</i> -NeuAc thioglycoside donor 206	58
Figure 13: 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN)	59
Figure 14: Literature 5-azido and 5-isothiocyanato NeuAc donors	67
Figure 15: Equatorially selective Pse and Leg donors	81
Figure 16: Naturally occurring Pse 27 and 30 containing differential nitrogen functionalities at C-5 and C-7	84
Figure 17: Literature Pse donors and the planned Pse donor 311	84
Figure 18: Pse donor 311 in its <i>tg</i> -conformation	100

LIST OF SCHEMES

Scheme 1: Biosynthetic pathway of legionaminic acid	15
Scheme 2: Biosynthetic pathway of pseudaminic acid	16
Scheme 3: Mechanism of a general glycosylation or sialylation reaction	18
Scheme 4: Gin's C-1 auxiliary mediated glycosylation using <i>N,N</i> -glycolyamide	19
Scheme 5: The general approach of C-3 auxiliary mediated glycosylation	19
Scheme 6: Sialylation approach by various modifications on <i>N</i> -5	20
Scheme 7: Tsvetkov's synthesis of legionaminic acid	22
Scheme 8: Seeberger's approach to synthesize a leg glycoside	23
Scheme 9: Whitfield's bioinspired synthesis of legionaminic acid	24
Scheme 10: Whitfield's further functionalization of Leg to provide a Leg donor	24
Scheme 11: Chen's chemoenzymatic approach to legionaminic acid	25
Scheme 12: Chemoenzymatic synthesis of Leg-5,7-di-N ₃ followed by glycosylations and deprotections	26
Scheme 13: Tsvetkov's approach to synthesize Pse5Ac7Ac	27
Scheme 14: Ito's synthesis of a pseudaminic acid and its glycoside	28
Scheme 15: Kiefel's synthesis of Pse5Ac7Ac starting from Neu5Ac	29
Scheme 16: Li's synthesis of a Pse donor and its glycosylation	30
Scheme 17: Glycosylation and deprotection of the Pse donor 130 giving Pse5,7Ac	30
Scheme 18: Glycosylation of the Pse donor 130 giving the trisaccharide 30	31
Scheme 19: Chou's chemoenzymatic synthesis of Pse	31
Scheme 20: Schoenhofen's one-pot enzymatic synthesis of CMP-Pse	32
Scheme 21: Kiefel's approach of oxidative deamination <i>en route</i> to Pse synthesis	33
Scheme 22: Crich's approach to oxidative deamination	34

Scheme 23: Kiefel's method of oxidation and reduction to invert configuration of C8	34
Scheme 24: Planned inversion of configuration replacing C-O with C-N at C7	35
Scheme 25: Kiefel's approach of C-9 deoxygenation	35
Scheme 26: General equilibria in the glycosylation of sialic acid with NIS/TfOH as promoter	36
Scheme 27: Retrosynthesis of a planned legionaminic acid donor	40
Scheme 28: Synthesis of the 5-N-Boc protected thioglycoside 162	41
Scheme 29: Overview of Kiefel's attempt to selectively functionalize C9-hydroxy	41
Scheme 30: Kiefel's successful functionalization of the C9-OH to give 166 and 122	42
Scheme 31: Selective trisylation of the C9 hydroxy group to give 168	42
Scheme 32: Attempts at the synthesis of 9-deoxy derivative of 168	43
Scheme 33: Deiodination of 170 and synthesis of 5-azido thioglycoside 174	44
Scheme 34: Selectivity reversal of 170 to give the 7,8-di-O-benzoate 176	45
Scheme 35: Inversion of configuration at C7 of 174	46
Scheme 36: Synthesis of the 5,7-diazido Leg donor 181 from 179	46
Scheme 37: Total synthesis of the Leg donor 181 from NeuAc, 3	47
Scheme 38: Synthesis of the sialic acid acceptor 186	48
Scheme 39: Glycosylation with Leg donor 181	49
Scheme 40: Deprotection of equatorial 2→6 galactosyl disaccharide to give 200	51
Scheme 41: Deprotection of equatorial 2→3 galactosyl disachharide to give 202	52
Scheme 42: Proof of regiochemistry of 195eq	52
Scheme 43: Original Zbiral deamination reaction with acetic acid and hydrazoic acid as nucleophiles	59
Scheme 44: Crich's alternate route to generate a <i>N</i> -nitrosamide intermediate in a modified Zbiral reaction	6

Scheme 45: Kiefel's one-pot protocol to generate <i>N</i> -nitrosamide in situ	60
Scheme 46: Modified Zbiral deamination with levulinic acid as nucleophile	61
Scheme 47: Zbiral deamination with isotopically labeled acetic acid as nucleophile	62
Scheme 48: Synthesis of the azido based 5- <i>epi</i> -NeuAc donor 206	62
Scheme 49: Synthesis of the 5- <i>O</i> -levulinoyl epimeric thioglycoside 224	63
Scheme 50: Alternative route to access 223 via deamination reaction with TfOH	63
Scheme 51: Electrophilic addition on alkenes to form triflate	64
Scheme 52: Generation of mannosyl triflate from the sulfoxide	64
Scheme 53: Glycosylation with the 5- <i>epi</i> -NeuAc donor 206	65
Scheme 54: Oscarson's stereoselective glycosylation with the KDO based 5- <i>epi</i> acetoxy donor 235	67
Scheme 55: Unverzagt's glycosylation reactions with various NeuAc donors	68
Scheme 56: Wong's glycosylations with 5-azido NeuAc donors 238 and 251	69
Scheme 57: Wong's glycosylations with the sialyl phosphite donor 242	69
Scheme 58: Li's glycosylations with the 5-azido NeuAc donor 239	70
Scheme 59: Lin's glycosylation with the armed thioglycoside donor 241	70
Scheme 60: Mukaiyama's glycosylation with the sialyl fluoride donor 243	71
Scheme 61: Synthesis of the 5-azido thioadamantanyl NeuAc donor 288	72
Scheme 62: Alternate route to access donor 288 via deamination reaction	72
Scheme 63: Glycosylation with the 5-azido NeuAc donor 288	72
Scheme 64: Synthesis of the equatorial thioglycoside donor 296	74
Scheme 65: Alternate route to access the donor 296 via deamination reaction	74
Scheme 66: Stereoselective glycosylation with the equatorial thioglycoside donor	75
Scheme 67: Deprotection protocol of the 5- <i>epi</i> azido glycosides 232 and 233	75

Scheme 68: Deprotection protocol of the 5-azido glycosides 204 and 290	77
Scheme 69: Equilibrating carbenium and bridged carbenium ions	78
Scheme 70: Ring contraction of a rhamnosyl 4- <i>O</i> -sulfonate	78
Scheme 71: Influence of C5 and C7 configuration on the side chain configuration	80
Scheme 72: Retrosynthetic analysis of the proposed Pse donor 311	88
Scheme 73: Synthesis of the 7- <i>O</i> -TBDMS thioglycoside 320	89
Scheme 74: Synthesis of the 8,9-diol derivative 321 and its selective trisylation	89
Scheme 75: Inversion of configuration at C-8 of 322	90
Scheme 76: Synthesis of the 9-deoxy sialoside 326	90
Scheme 77: Attempted late stage oxidative deamination with levulinic acid	91
Scheme 78: Synthesis of the 7- <i>O</i> -Naphthyl thioadamantyl sialoside 330	92
Scheme 79: Oxidative deamination of 330 with levulinic acid as nucleophile	93
Scheme 80: Selective trisylation of 9-hydroxy group via dibutyltin acetal derivative	94
Scheme 81: Inversion of configuration at C8 of 335	94
Scheme 82: Synthesis of the 9-deoxy sialoside	95
Scheme 83: Deprotection of the naphthyl and levulinoyl groups to access 312	95
Scheme 84: Synthesis of the 5,7-diazido Pse donor 311	96
Scheme 85: Total synthesis of the Pse donor 311	97
Scheme 86: Glycosylation reaction with the Pse donor 311	98
Scheme 87: Deprotection of 343 to access Pse5Ac7Ac, 2	101
Scheme 88: Kiefel's regioselective functionalization of the azides in 121	102
Scheme 89: Regioselective reduction of azide in the deprotection of 351	102
Scheme 90: Alternate regioselective reduction of azide in the deprotection of 351	108

LIST OF ABBREVIATIONS

Ac	Acetyl
Ada	Adamantyl
Ar	Aryl
ATP	Adenosine triphosphate
AWMS	Acid washed molecular sieves
Boc	<i>tert</i> -Butoxycarbonyl
Bn	Benzyl
Bz	Benzoyl
Cbz	Benzyloxycarbonyl
CMP	Cytidine-5-monophosphate
CSA	Camphorsulfonic acid
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIAD	Diisopropyl azodicarboxylate
DIPEA	Diisopropylethylamine
DMAP	4-(Dimethylamino)-pyridine
DMF	Dimethyl formamide
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
ESIHRMS	Electrospray ionization high resolution mass spectroscopy
Equiv	Equivalent
Et	Ethyl

Gal	Galactose
GalNAc	<i>N</i> -Acetyl galactosamine
<i>gg</i>	gauche-gauche
<i>gt</i>	gauche-trans
GDP	Guanosine diphosphate
GlcN	Glucosamine
h	Hour
HPLC	High performance liquid chromatography
Hz	Hertz
KDN	Keto deoxy nonulosonic acid
KDO	Keto deoxy octulosonic acid
Leg	Legionaminic acid
LPS	Lipopolysaccharide
LNnt LOS	Lacto- <i>N</i> -neotetraose lipooligosaccharide
MAG	Myelin associated glycoprotein
Me	Methyl
MS	Molecular sieves
Mp	Melting point
mmol	Millimole
MS	Molecular sieves
Neu5Ac	5-Acetylneuraminic acid
Neu5Gc	5-Glycolylneuraminic acid
NAD	Nicotinamide adenine dinucleotide

Nap	Naphthyl
NBz	<i>p</i> -Nitrobenzyl
NBS	<i>N</i> -Bromosuccinimide
NIS	<i>N</i> -Iodosuccinimide
NMR	Nuclear magnetic resonance
NulO	Nonulosonate
nOe	Nuclear overhauser effect spectroscopy
PEP	Phosphoenol pyruvate
PG	Protecting group
Ph	Phenyl
Piv	Pivoloyle
PMP	<i>p</i> -Methoxybenzyl
ppm	Parts per million
Pse	Pseudaminic acid
pTSA	4-Toluene sulfonic acid
Py	Pyridine
rt	Room temperature
Sia	Sialic acid
Siglec	Sialic acid-binding immunoglobin-like lectin
SFORD	Single frequency off resonance coupling
Stick's reagent	Imidazole-1-sulfonyl azide
sTn	Sialyl Tn antigen (sia eq.(2→6)galNAc)
TBAF	Tetrabutylammonium fluoride

TBDMS/TBS	<i>tert</i> -Butyldimethylsilyl
TBSCl	<i>tert</i> -Butyldimethylsilyl chloride
TBSOTf	<i>tert</i> -Butyldimethylsilyl triflate
TEA	triethylamine
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
<i>tg</i>	trans-gauche
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Tol	Toluenyl
TPSCl	2,4,6-triisopropylbenzenesulfonyl chloride
Troc	2,2,2-Trichloroethoxycarbonyl
UPD	Uridine diphosphate

CHAPTER 1. INTRODUCTION

1.1 Sialic acids

Sialic acids are the 9-carbon keto sugar acids found at the terminal, non-reducing end of various glycoproteins and glycolipids that are involved in a wide range of biological processes.¹⁻⁵ To date, 43 different sialic acids have been discovered of which the most common is *N*-acetylneuraminic acid (Figure 1), which is found in various glycosidic linkages such as equatorial (2→3) and equatorial (2→6) linkages to galactose and equatorial (2→8) or equatorial (2→9) linkages in polysialic acids. Therefore, *N*-acetylneuraminic acid itself is considered to be the parent sialic acid, whereas the other members of the family are treated as its derivatives.

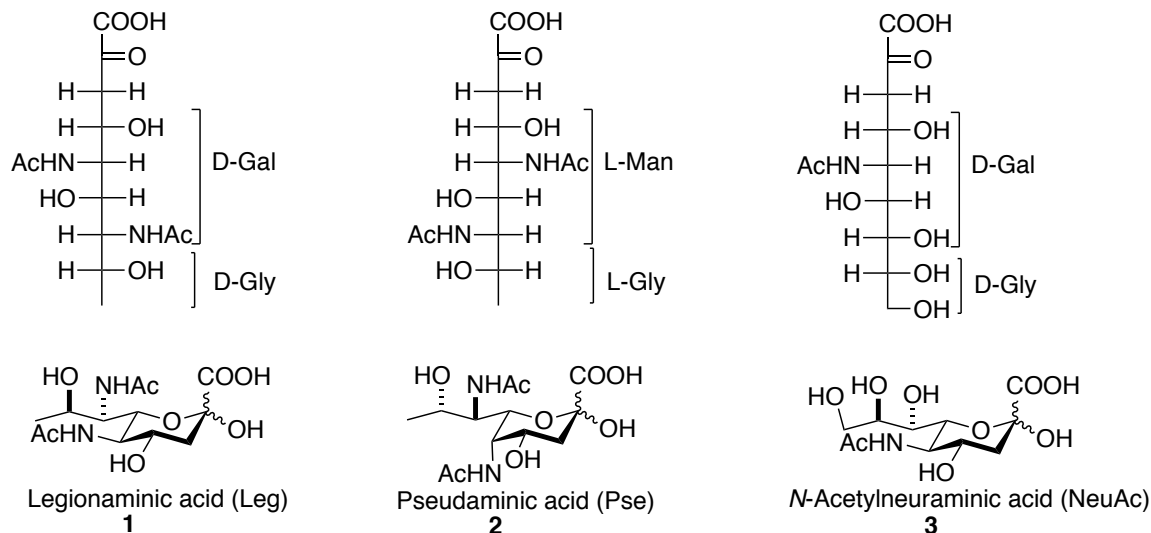


Figure 1: Fischer projections and cyclic structures of legionaminic acid, pseudaminic acid and *N*-acetylneuraminic acid

1.2 Biological roles of sialic acids

Sialic acids, one of the most diverse classes of sugar, are found on the outermost glycan chains of glycoproteins and glycolipids. On the positive side they perform important functions and are necessary for normal development,⁶⁻⁷ while on the negative

side they are frequently the sites that pathogens recognize and attach to.⁸⁻¹⁴ Sialic acids link to the preceding sugar in a glycan from the 2-position, and can carry different substituents at the 4,5,7,8 and 9-positions, which is the primary reason for their diversity.^{9,10} They are present at the outermost position of glycans leading to their diversity being utilized by a variety of sialic acid binding proteins of many bacterial and viral pathogens.⁷ Additionally, sialic acids are recognized by intrinsic receptors in vertebrate cells such as the selectins and the siglecs.^{10,14} The pathological significance of sialic acid diversity can be understood by the following three examples.

- 1) Avian influenza viruses, also called 'bird flu', preferentially recognize sialic acids that are equatorial (2→3) linked to the glycan chains. Fortunately, humans display equatorial (2→6) linked sialic acids on the epithelium of the upper airways and consequently are resistant to such viruses. Therefore, the avian influenza virus must undergo certain mutations of the sialic acid-binding region of the virus hemagglutinin to infect humans. This is the case in the intermediate hosts like pigs, where both equatorial (2→3) and equatorial (2→6) linked sialic acids are present on the airways.¹⁵ Chimpanzees, our closely related great apes, have been found to be devoid of equatorial (2→6) linked sialic acids in the airway. This is the reason for the resistance of chimpanzees to human influenza A infection.¹⁶
- 2) The addition of 9-*O*-acetyl esters to the side chain of sialic acids has two contradictory effects. On one hand, this can block the binding of the influenza A virus; on the other hand, this modification is required for binding of common cold viruses like influenza C and certain coronaviruses, which make up about a third of human common cold viruses.^{8,12} This finding helped to find a detector for the

presence of 9-*O*-acetylated sialic acids *in situ* by developing recombinant soluble forms of the influenza C hemagglutinin.^{12,17}

- 3) Merozoites of *Plasmodium falciparum*, governed by different proteins, invade human red blood cells by binding with the sialic acids. Our closely related great apes, chimpanzees are susceptible to *Plasmodium reichenowii* malarial parasites instead of *Plasmodium falciparum*, largely due to the differential preference of the parasite binding proteins for human and nonhuman sialic acids.¹⁸ This can be explained as an adaptation that humans developed to deal with the *P. reichenowii* infection, only to be infected later by *P. falciparum*.

Some examples of the naturally occurring sialosides with various linkages are shown here (Figure 2).

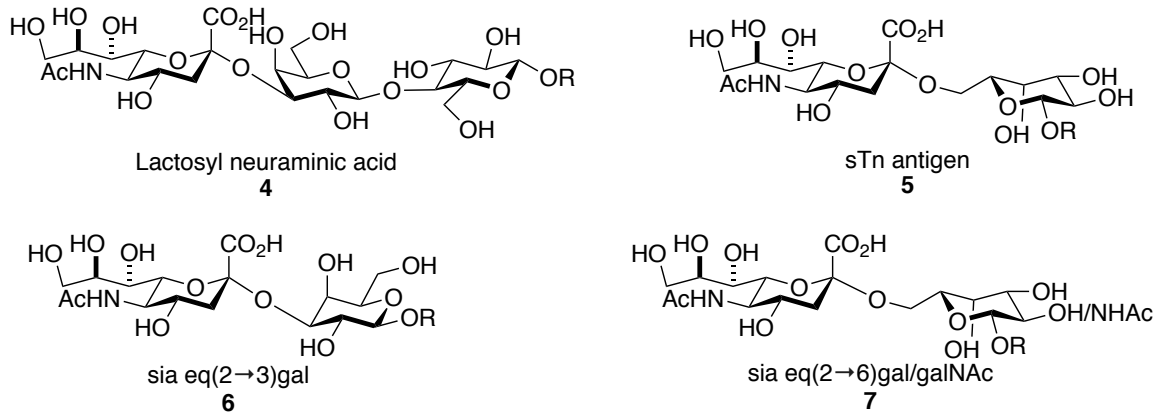


Figure 2: Naturally occurring sialoside linkages

In bacteria, sialic acids are found on glycoconjugates such as capsular polysaccharides (K-antigens) and lipopolysaccharides (O-antigens). Sialic acid expressing bacteria are the cause of various harmful diseases in humans, such as *Escherichia coli* strain K1, *Neisseria meningitides* group B,C (meningitis),¹⁹ and *Camphylobacter jejuni* (food-borne gastroenteritis).²⁰ Sialic acids on the cell surface of

these bacteria provide a protective barrier that enables bacteria to evade detection and attack by the host's immune system.

Some bacterial pathogens require sialic acids to interact with their host cells. The lectins involved, typically called adhesins, are attached to the bacterial surface. *Helicobacter pylori*, expresses two different adhesins which can recognize sialic acid, and causes peptic ulcers in humans.^{21,22} *E. coli* strain K99 causes lethal dysentery among animals;^{23,24} the adhesins expressed by it show high specificity toward the Neu5Gc-equatorial(2→3)Gal-axial(1→4)Glc structure on glycolipids.²⁵ *Vibrio cholerae* causes cholera toxin, and is an example of soluble sialic acid-binding lectins.²⁶ Its B subunits show specific binding to a sialylated glycolipid (ganglioside GM1), which delivers the A subunit to the cytosol. This results in overproduction of adenylate cyclase, producing cyclic AMP in gastrointestinal epithelial cells ultimately causing severe diarrhea. Other sialic acid bearing toxins are *Clostridium tetani*, that causes tetanus, and *Clostridium botulinum*, that causes botulism.

The legionaminic acid and pseudaminic acids are deoxy acetamido analogues of *N*-acetylneuraminic acid found in microorganisms (Figures 1 and 3). These two derivatives are quite common and significant given the fact that their glycosides are found in lipopolysaccharides and glycoproteins of multiple Gram-negative bacteria including *Pseudomonas aeruginosa*, *Legionella pneumophila* serogroup 1, *Camphylobacter jejuni*, and *Camphylobacter coli*.²⁷⁻²⁹ The equatorial glycosides of NeuAc and Leg are considered to be α -glycosides, whereas the equatorial glycosides of Pse are considered to be β -glycosides. This is based on the Rosanoff nomenclature convention, which links the anomeric configuration to the configuration of the

stereogenic center at the bottom position of the Fischer projection (Figure 1).³⁰ Therefore, in order to avoid any confusion, in this thesis, all glycosides will be simply referred as equatorial or axial glycosides.

1.3 Legionaminic acid and its biological roles

Legionaminic acid was originally characterized in 1994 from the lipopolysaccharide (LPS) serotype 1 O-chain of *Legionella pneumophila*, the Gram-negative bacterium responsible for Legionnaires' disease, where it is present as a 5-*N*-acetimidoyl-7-*N*-acetyl-legionaminic acid homopolymer with equatorial-2→4-linkages.³¹ Legionnaire's disease, a type of pneumonia, is acquired by breathing mist from water that contains the *Legionella pneumophila* bacteria, typically from hot tubs, showers or air-conditioning units of large buildings. The pneumonia is chronic and often fatal due to the fact that *Legionella pneumophila* replicates within the alveolar macrophages of the human lung tissues.³² Legionaminic acid has since been reported in various bacterial glycoconjugates including the repeating unit of heteropolymeric LPS, capsular polysaccharides, and O-linked monosaccharide modifications on the flagellae of *Camphylobacter jejuni*.^{33,34} Though it is structurally homologous to Neu5Ac, the role of legionaminic acid in the physiology of bacteria and its impact on host-pathogen interaction is largely unknown.³⁵ Studies examining the biology of Leg5,7Ac₂ have been critically hindered by the low availability of this sugar. The existing synthetic routes for Leg5,7Ac₂ are highly demanding and low yielding.

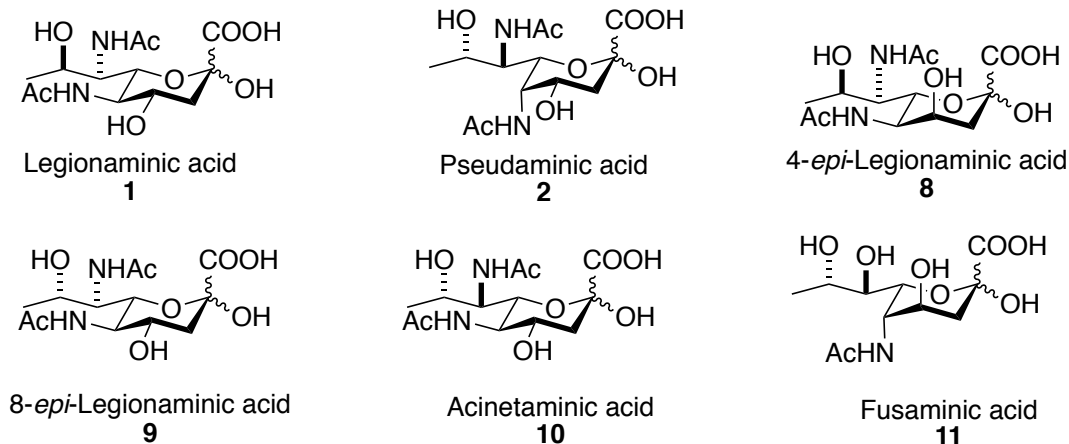


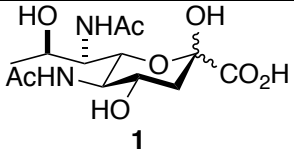
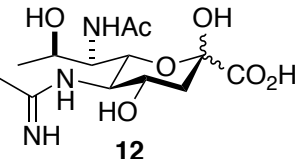
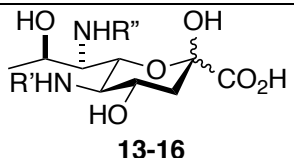
Figure 3: Naturally occurring bacterial nonulosonate (NuLO) sugars.

Legionaminic acids belong to the growing family of bacterial nonulosonate (NuLO) sugars, of which currently there are six known members: legionaminic acid, 4-*epi*-legionaminic acid, 8-*epi*-legionaminic acid, pseudaminic acid, acinetaminic acid, and fusaminic acid (Figure 3).^{27, 36-39} All are 9-carbon keto acid sugars and therefore resemble sialic acid, but differ in being deoxy at C9. Also, excluding fusaminic acid, all contain various *N*-acyl substituents at C7 instead of a hydroxyl group. It has been observed that there is much greater diversity in their C5 and C7 *N*-acyl substituents than is found in *N*-acetylneuraminic acid itself. The legionaminic acid Leg5,7Ac₂ is structurally homologous to *N*-acetylneuraminic acid (Neu5Ac, sialic acid) in having the same configuration, with the differences at C9, with methyl group instead of -CH₂OH, and at C7, with an acetamido group instead of a hydroxyl. Therefore, if Neu5Ac is replaced with Leg5,7Ac₂ on the termini of glycoconjugates, biological interactions that depend upon the glycerol or exocyclic moiety will be affected. For example, studies on a fluorescently labeled lactosyl derivative have shown that susceptibilities to sialidases are greatly reduced by the presence of Leg5,7Ac₂ instead of Neu5Ac.⁴⁰

Siglecs are defined as sialic acid-binding lectins and are another class of Neu5Ac interacting molecules whose biological activity is influenced by the sialic acid analogs. GD1a, a di-sialylated ganglioside, acts as a ligand for the glycoprotein (MAG or Siglec-4) associated with myelin. Synthesis of a form of GD1a containing Leg5,7Ac₂ was carried out and the product showed no interaction with Siglec-4,⁴¹ showcasing the importance of the glycerol or exocyclic moiety on the biological properties of NulOs. Modification of glycans on therapeutic glycoproteins can also change their biological activities. Previously, the best approach was to develop resistance for the sialidase cleavage, but the recent finding of natural antibodies in human sera has shown the exchange with Leg5,7Ac₂ is beneficial and enhances serum half-life. For example, it was found that exchanging NeuAc with Leg5,7Ac₂ counteracts the serum protection afforded to *Neisseria gonorrhoeae* by Neu5Ac. *N. gonorrhoeae* modifies its cell surface lacto-*N*-neotetraose lipooligosaccharide (LNnT LOS) molecules with Neu5Ac, utilizing host-derived CMP-Neu5Ac and its sialyltransferase Lst. This is the primary mechanism by which the organism evades complement-mediated killing and survives in the host. However, when *N. gonorrhoeae* is treated with CMP-Leg5,7Ac₂, these cells modify their surface LNnT LOS with Leg5,7Ac₂ and are then prone to complement-mediated killing. In the light of this finding, CMP-Leg5,7Ac₂ is now being considered and evaluated for its therapeutic potential in the treatment of *N. gonorrhoeae* infections.⁴²

The structures of legionaminic acid found in different pathogenic bacteria are summarized in the Table 1.

Table 1: Structures of legionaminic acids found in different pathogenic bacteria

Legionaminic acid	Bacterial source	Reference
 <p style="text-align: center;">1</p>	<i>Vibrio alginolyticus</i> 945-80 <i>Acinetobacter baumannii</i> O24 <i>Vibrio parahaemolyticus</i> O2 <i>Campylobacter jejuni</i> 11168 <i>Escherichia coli</i> O161	43,44 43,45 46,47 33 48
 <p style="text-align: center;">12</p>	<i>Legionella pneumophila</i> serogroup 1 <i>Pseudomonas fluorescens</i> ATCC 49271 <i>Vibrio salmonicida</i> NCMB 2262 <i>Campylobacter coli</i> VC167 <i>Campylobacter jejuni</i> 11168	31,43 43,49 43,50 34 33
 <p style="text-align: center;">13-16</p> <p>R' = C(=O)CH₂CH(OH)CH₃; R'' = Ac R' = Ac; R'' = C(=O)CH(NHAc)CH₃ R' = CH(NHCH₃)CH₃; R'' = Ac R' = C(=O)CH₂CH₂CH(CO₂H)NHCH₃; R'' = Ac</p>	<i>Acinetobacter baumannii</i> O24 <i>Vibrio parahaemolyticus</i> KX-V212; <i>Escherichia coli</i> O161 <i>Campylobacter coli</i> VC167 <i>Campylobacter jejuni</i> 11168 <i>Clostridium botulinum</i>	43,45 46,48 34 33 51

1.4 Pseudaminic acid and its biological roles

Pseudaminic acid (Pse5Ac7Ac) was first discovered by Knirel and co-workers in the lipopolysaccharides of *Pseudomonas aeruginosa* O7/O9 and *Shigella boydii* type 7 in 1984.⁵² The structure was found to be 5,7-diacetamido-3,5,7,9-tetra-deoxy-L-glycero-L-manno-non-2-ulosonic acid, which was a nonulosonic acid but not a direct derivative of neuraminic acid. This opened a whole new chapter of the family belonging to pseudaminic acid that differed in the *N*-acyl functionality at C-5 and C-7. In comparison with *N*-acetyl neuraminic acid, Pse contains one more amido group (on C7) and one less

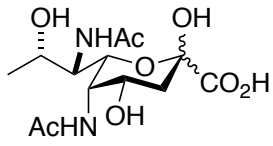
hydroxyl group (on C9), together with the opposite stereochemistry at C5, C7 and C8. The two amido groups have been found to be quite diverse, ranging from acetyl to 3-hydroxybutyryl and formyl groups, further increasing the structural complexity of Pse. Pseudaminic acid containing glycan structures have been isolated from a large number of pathogenic bacteria, wherein the bacterial proteins are glycosylated with pseudaminic acid via a very common and essential post-translational modification. Such types of post-translational modification, commonly called protein glycosylation, is quite common in eukaryotes and helps increase the functional and structural diversity of proteins. However, bacterial protein glycosylation is a recently discovered phenomena, and has significantly helped in the study of bacterial pathogenesis and in the development of therapeutic and diagnostic tools.⁵³⁻⁵⁶ Unique to bacteria, the pseudaminic acids have been found to be important components of cell surface glycans (such as lipopolysaccharide O-antigens, and capsular polysaccharides) and glycoproteins (such as pilins and flagellins) which are associated with the bacterial virulence.^{28,57} In glycoproteins, pseudaminic acid uses two amino acids Ser and Thr (in case of *Helicobacter pylori* and *Campylobacter jejuni*) or other glycans (in case of *P. aeruginosa*) to link with the peptide backbone. *Pseudomonas aeruginosa*, in particular, is a notorious pathogen which has been linked to cystic fibrosis and wide varieties of gastrointestinal diseases. The importances of sialic acids in eukaryotes are pretty well characterized and therefore, there is no doubt about their importance in bacteria.^{37,58} Unfortunately, the exact biological role of pseudaminic acid in bacterial glycoproteins remains understudied, largely due to the unavailability of homogeneous pseudaminic acid and its derivatives. Therefore, an efficient synthesis of

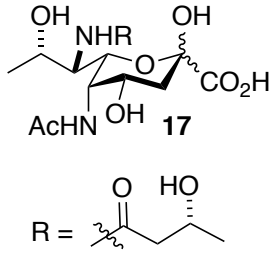
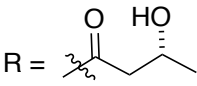
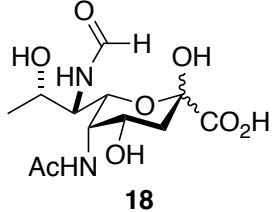
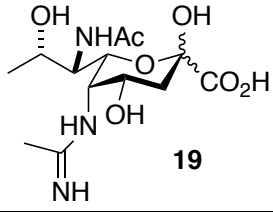
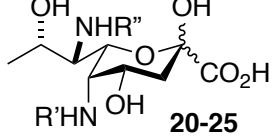
Pse and its derivatives will not only pave the way to study its biological roles, but also will be significant in developing therapeutic and diagnostic vaccines.

Post-translational modification with pseudaminic acid is necessary for functional flagella and this glycosylation has been considered important for motility in bacteria. Liu *et al.*⁵⁹ attempted to verify this phenomenon via glycoengineering analysis. They supplemented exogenous azide-labelled pseudaminic acid biosynthetic precursors in a *C. jejuni* strain that lacked the biosynthetic enzymes for pseudaminic acid synthesis. The results showed that the motility was restored in this *C. jejuni* strain. The authors were also able to perform different chemical biology analyses of this incorporation using the biotin tag. Kaewsapsak *et al.*⁶⁰ utilized azide-labeled glycosylated proteins in *H. pylori* to ligate immune stimulants, which helped promote immunological destruction of bacteria carrying pseudaminic acid. Menard *et al.*⁶¹ also recently discovered three small molecule inhibitors of the enzyme PseB which could penetrate the cell membrane of Gram-negative bacteria preventing the formation of flagella.

The structures of pseudaminic acid found in different pathogenic bacteria are summarized in the Table 2.

Table 2: Structures of pseudaminic acids found in different pathogenic bacteria

Pseudaminic acid	Bacterial source	Ref.
 <p style="text-align: center;">2</p>	<i>Escherichia coli</i> O136	62
	<i>Proteus vulgaris</i> O39	63
	<i>Campylobacter jejuni</i> 81-176	64,65
	<i>Campylobacter jejuni</i> 11168	33
	<i>Campylobacter coli</i> VC167	66
	<i>Pseudoaeromonas atlantica</i> T9	52
	<i>Helicobacter pylori</i> 1061	65,67
	<i>Pseudoalteromonas atlantica</i> IAM 14165	68
	<i>Aeromonas caviae</i> UU51	65
	<i>Rhizobium sp.</i> NGR234	69
	<i>Sinorhizobium meliloti</i> Rm1021	70,71

	<i>Cellulophaga fucicola</i>	72
	<i>Vibrio vulnificus</i> 27562	73
	<i>Piscirickettsia salmonis</i>	74
 <p>17</p> <p>R = </p>	<i>Pseudomonas aeruginosa</i> O10a <i>Shigella boydii</i> type 7 <i>Pseudomonas aeruginosa</i> O9a, 9b <i>Sinorhizobium fredii</i> HH103 <i>Kribbella</i> spp. VKM <i>Actinoplanes utahensis</i> VKM Ac-674	52 75,76 76,77 78 79,80 79,81
 <p>18</p>	<i>Pseudomonas aeruginosa</i> O7a, 7b, 7d and O7a, 7d <i>Pseudoalteromonas distincta</i> KMM 638	82,83 84
 <p>19</p>	<i>Vibrio cholera</i> O:2 <i>Campylobacter jejuni</i> 81-176 <i>Campylobacter coli</i> VC167	85 64,65 66
 <p>20-25</p> <p>R' = Ac; R'' = glycate R' = C(=O)CH₂CH(OH)CH₂; R'' = C(=O)H R' = R'' = C(=O)CH(OH)CH₂OH R' = Ac; R'' = C(=NH)CH₃ R' = C(=O)CH(OCH₃)CH₂OCH₃; R'' = C(=NH)CH₃ R' = C(=O)CH(OCH₃)CH₂OCH₃; R'' = Ac</p>	<i>Vibrio vulnificus</i> 27562 <i>Pseudomonas aeruginosa</i> O7a, 7b, 7c <i>Campylobacter jejuni</i> 81-176 <i>Campylobacter jejuni</i> 11168 <i>Campylobacter jejuni</i> 11168 <i>Campylobacter jejuni</i> 11168	73 83 64 33 33 33

1.5 Structure and linkage diversity in legionaminic and pseudaminic acid

Legionella pneumophila, a Gram-negative bacterium, is the causative agent of Legionnaires' disease in whose serotype 1 O-chain lipopolysaccharide legionaminic acid is found in the form of the equatorially linked homopolymer **26**.³¹ Pseudaminic acid is

found linked equatorially in the lipopolysaccharide **27** of *Pseudomonas aeruginosa* O10 (Figure 4).⁷⁷

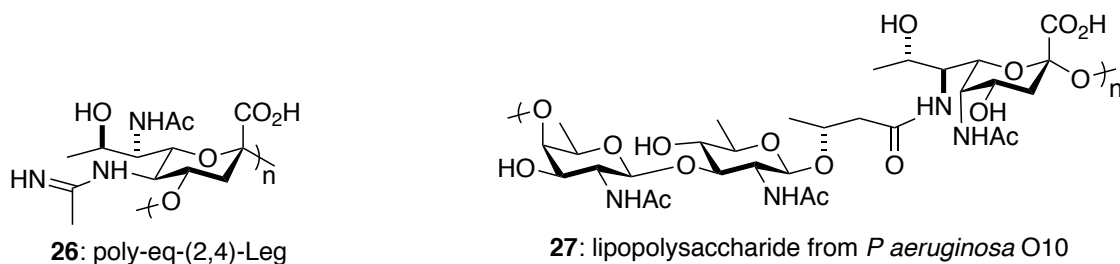


Figure 4: Structures of polylegionaminic acid and the O-specific lipopolysaccharide from *P. aeruginosa* O10

In 2012, Kandiba *et al.*⁸⁶ discovered a 5-*N*-formyl derivative of legionaminic acid from the glycan of a haloarchaeal pleomorphic virus (HRPV-1). The glycan was found to be the pentasaccharide **28** containing a terminal 5-*N*-formyl-legionaminic acid residue alongside glucose, glucuronic acid, mannose and sulphated glucuronic acid (Figure 5).

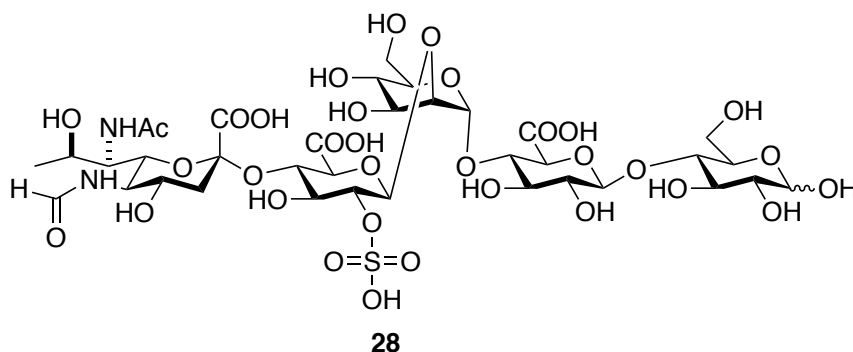


Figure 5: Structure of a glycan from HRPV-1 containing legionaminic acid

Naturally occurring pseudaminic acids and their derivatives have often been found to be linked via the amines either at C-5 or C-7. Such example is the C-7 linked pseudaminic acid derivative found in *Kribella spp* (Figure 6).⁵⁷

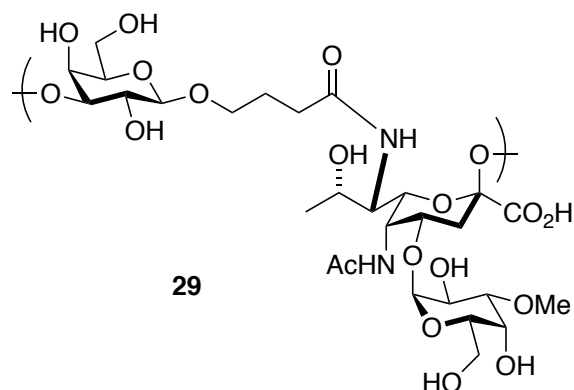


Figure 6: Structure of Teichulosonic acid fragment in *Kribella sp.* containing pseudaminic acid

Among the Pse-containing glycans and glycoconjugates, the *P. aeruginosa* 1244 pilin glycan trisaccharide **30** (Figure 7) is of particular importance.

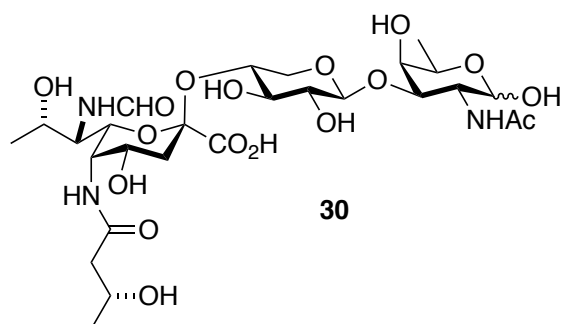


Figure 7: Structure of the *P. aeruginosa* 1244 pilin containing pseudaminic acid

P. aeruginosa is a Gram-negative pathogen known to have resistance to many antibacterial agents and hard to be detected by our immune system due to biofilm formation.⁸⁷ It causes cystic fibrosis a potentially mortal infection in patients with weak immune systems. The glycosylated pilin contains the *P. aeruginosa* 1244 strain, which has been isolated and used in pilus-mediated adhesion studies.⁸⁸ The trisaccharide is glycosylated to D-fucosamine by the pilin at the C-terminal residue through an equatorial-glycosidic linkage.⁸⁹ This is mediated by an oligosaccharyltransferase, the pilO gene product, which transfers the trisaccharide from the carrier lipid to the pilin.⁹⁰ Organized

pili from the pilin play an important role in the bacterial pathogenesis. Glycosylated *P. aeruginosa* 1244 was found to colonize the lung tissue 3 times more efficiently than the non-glycosylated *P. aeruginosa* 1244,⁹¹ and is a vital tool in immunogenicity. The trisaccharide **30** glycoconjugate can be considered as a candidate for a potential vaccine, as it protected immunized mice from *P. aeruginosa*.⁹²

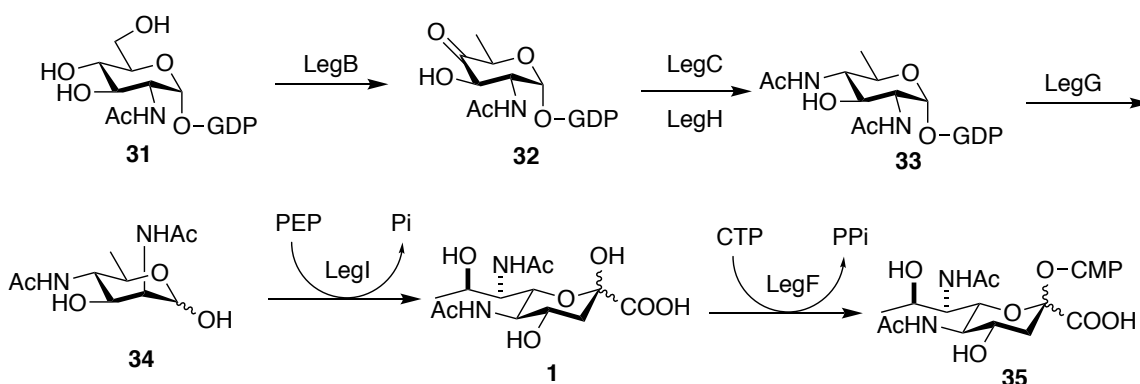
1.6 Biosynthesis of legionaminic acid and pseudaminic acid

L. pneumophila has been found to contain a homopolymer of Leg as a distal part of its lipopolysaccharide (LPS). Studies have found that a segment of the *Legionella* genome involved in the biosynthesis of LPS contains putative genes similar to three genes involved in the biosynthesis of Neu5Ac, GlcNAc-6-phosphate 2-epimerase, Neu5Ac synthetase and CMP-Neu5Ac synthetase. Therefore, it was concluded that the biosynthesis of legionaminic acid is similar to that of Neu5Ac.^{10,93}

In 2009, the biosynthetic pathway for Leg5,7Ac₂ in *Campylobacter jejuni* was elucidated.⁹⁴ Unexpectedly this pathway used GDP-GlcNac (Scheme 1) as the key building block, unlike related nonulosonic acid pathways, which use UDP-GlcNac.

The first step in the biosynthesis of legionaminic acid is catalyzed by LegB an NAD-dependent 4,6-dehydratase, which eliminates water from C4 and C6 of GDP-*N*-acetyl-glucosamine **31** to give the 4-keto intermediate, GDP-2-acetamido-2,6-dideoxy- α -D-xylo-hexos-4-ulose **32**. Compound **32** was then converted to GDP-4-amino-4,6-dideoxy- α -D-GlcNAc with the help of LegC, a PLP-dependent aminotransferase, which is further converted to GDP-2,4-diacetamido-2,4,6-trideoxy- α -D-glucopyranose (**33**) by LegH, an *N*-acetyltransferase. The enzyme LegG, acting as an NDP-sugar hydrolase and 2-epimerase, catalyzes conversion of the intermediate **33** into a 6-deoxy-mannose

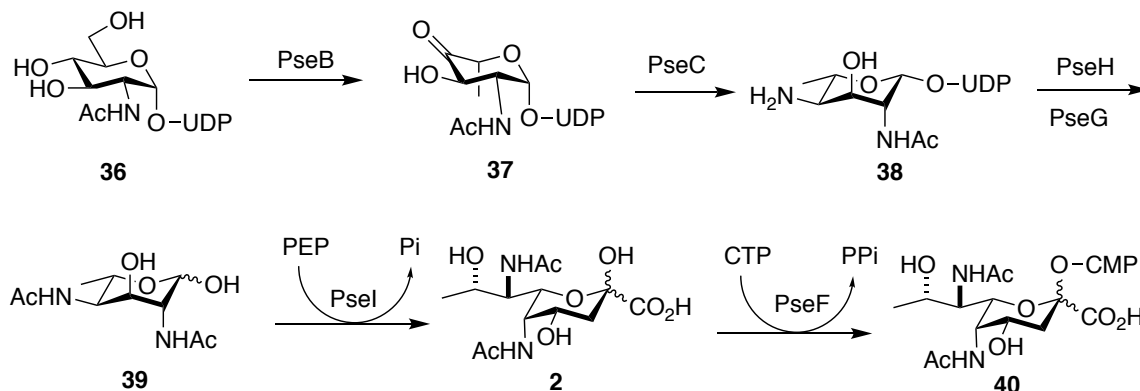
derivative (or a D-rhamnose sugar), 2,4-diacetamido-2,4,6-trideoxy-D-mannopyranose **34**. Aldol condensation with phosphoenolpyruvate with the help of enzyme LegI, a legionaminic acid synthase, then converts compound **34** to legionaminic acid **1** (Scheme 1). The so-synthesized legionaminic acid must be nucleotidylated as the CMP-linked derivative for incorporation into glycans.¹⁰ Legionaminic acid is CMP-activated by the enzyme LegF, a CMP-legionaminic acid synthetase, giving CMP-legionaminic acid **35** (Scheme 1).



Scheme 1: Biosynthetic pathway of legionaminic acid

Both pseudaminic acid and legionaminic acid are biosynthesized from a common precursor, *N*-acetyl-glucosamine. The complete biosynthesis of pseudaminic acid was elucidated in *Campylobacter jejuni* and *Helicobacter pylori*, where pseudaminic acid is obtained in five steps from UDP-*N*-acetylglucosamine (Scheme 2).^{34,95-97} The initial conversion of first step of converting UDP- α -D-GlcNAc **36** to UDP-2-acetamido-2,6-dideoxy- β -D-arabino-hexos-4-ulose **37** is catalyzed by a dual functionality enzyme, PseB which acts as both an NAD(P)-dependant dehydratase and a C-5 epimerase.⁹⁷⁻¹⁰⁰ The second enzyme, PseC, then converts compound **37**, a D-sugar into UDP-4-amino-4,6-dideoxy- β -L-AltNac **38**, an L-sugar.^{96,97,101} The intermediate **38** is then converted to 2,4-diacetamido-2,4,6-trideoxy- β -L-altropyranose **39** with the help of two enzymes PseH and

PseG.^{102,103} Thereafter, an aldol condensation of compound **39** with phosphoenolpyruvate (PEP) takes place in the presence of the enzyme PseI, giving the pseudaminic acid **2** (Scheme 2).^{62,97,98} For incorporation into glycans, pseudaminic acid is converted to its derivative by the enzyme PseF, a CMP-pseudaminic acid synthetase, which utilizes CTP, giving CMP-pseudaminic acid **40** and pyrophosphate (Scheme 2).^{97,98}



Scheme 2: Biosynthetic pathway of pseudaminic acid

All the aforementioned enzymes have been now thoroughly studied and characterized. Unfortunately, it was discovered that the first enzyme in the pathway, PseB inhibits CMP-pseudaminic acid pathway at a micromolar level. Therefore, huge doses of the natural substrate UDP-GlcNAc **36** are required to push this pathway forward.^{97,98} The structure of the fourth enzyme of the pathway PseG was fully characterized by X-ray crystallography in 2009, proving that PseG was a UDP-hydrolase belonging to a superfamily of inverting glycosyltransferases.¹⁰³ Even after this revelation, Liu and Tanner¹⁰² showed that PseG wasn't the CMP-pseudaminyltransferase responsible for linking CMP-activated pseudaminic acid **40** into glycan structures.

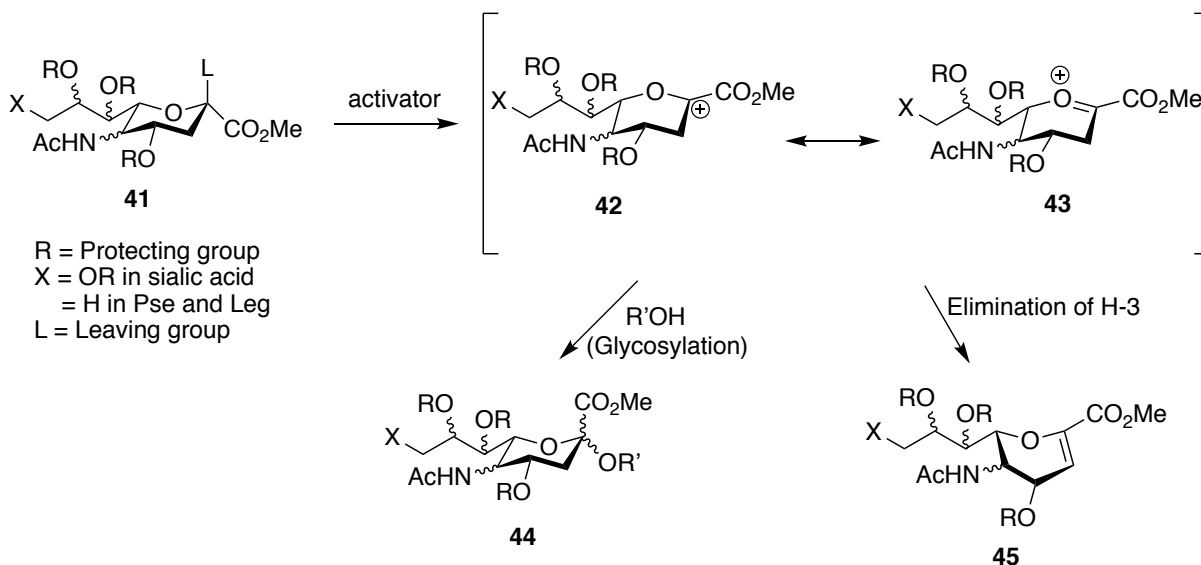
Exactly how the possible glycosyltransferase(s) are responsible for the incorporation of these CMP-linked nonulosonates into glycan structures is not known. Recently, the glycosyltransferase responsible for the transfer of CMP-activated

pseudaminic acid **40** onto the flagellin of *Aeromonas caviae* has been discovered.¹⁰⁴ Although direct evidence of the motility-associated factor *mafI* gene providing the enzyme responsible for adding pseudaminic acid onto the flagellin was not provided, the studies showed that *mafI* is a pseudaminyl transferase. As the first report identifying a probable pseudaminyl transferase, it can be expected that this will help in identifying the presence of similar glycosyltransferases in other bacteria.

1.7 Synthesis of sialic acid glycoconjugates

The universe of the sialic acids including legionaminic acid and pseudaminic acid, their diverse linkages and biology is defined as the sialome.¹⁰⁵ Unfortunately, nature produces sialosides in micro heterogeneous form and in small concentrations, thus the development of efficient chemical or enzymatic methods for their synthesis is in great need.¹⁰⁶ Enzymatic or chemoenzymatic pathways, though providing a solution for the synthesis of sialyl glycoconjugates still suffer from scale-up issues. Therefore, the chemical approach is the only solution to access natural sialyl glycoconjugates in sufficient quantity to study, and to gain access to non-natural linkages. The naturally occurring sialosides, excluding legionaminic and pseudaminic acid, are all equatorially linked. Obtaining the equatorial anomer in preference to the axial one in glycosylation is challenging. There are several reasons for this beginning with the high reactivity of typical sialyl donors. The unsubstituted nature of C-3 also aids to the formation of the axial glycoside. Additionally, the formation of equatorial glycoside requires the avoidance of the anomeric effect and cannot depend on stereodirecting participation from

C-3 due to absence of the functional group. All of these reasons contribute to the generally generally low yields and poor selectivity observed in the glycosylation reactions of sialic acid donors.



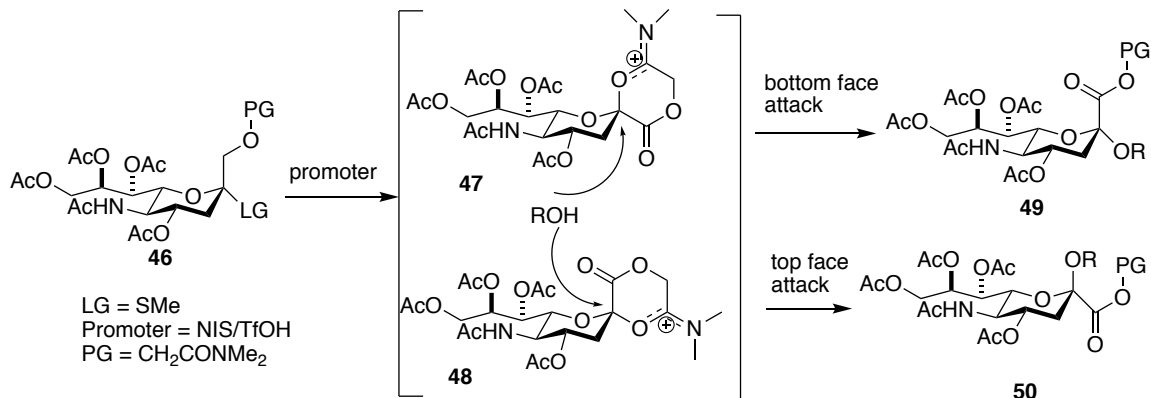
Scheme 3: Mechanism of a general glycosylation or sialylation reaction

Because of all these complications, the development of methodology for the synthesis of equatorial sialyl glycosides has been a major focus in synthetic carbohydrate chemistry. A variety of approaches have been applied to overcome this problem, and can be broadly classified into three main groups: 1) Sialylation assisted by auxiliary groups; 2) Conversion of acetamide at C-5 to more electron withdrawing groups; and 3) Use of cyclic protecting groups.

1.7.1 Sialylation assisted by auxiliary groups

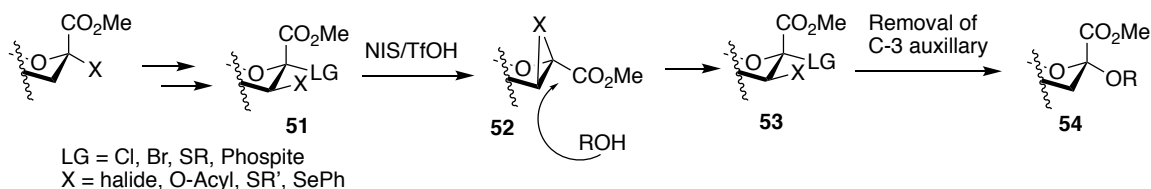
Glycosylation assisted by the neighboring groups is a popular and common method applied to the control of stereoselectivity.¹⁰⁷ In sialyl donors, incorporation of an auxiliary groups at the C-1 or C-3 positions is a common theme and has led to the improvement of the glycosylation selectivity. Two groups have studied the C-1 auxiliaries, those of Gin (Scheme 4)¹⁰⁸ and Takahashi¹⁰⁹ utilizing 2-thioethyl ester and

N,N glycolamide auxiliaries, respectively.



Scheme 4: Gin's C-1 auxiliary mediated glycosylation using *N,N*-glycolamide

Gin's *N,N*-dimethylglycolamide auxiliary assisted sialylation (Scheme 4) was selective for primary alcohols, but lost its selectivity with the less reactive secondary alcohol acceptors.¹⁰⁸ Takahashi's thioether auxiliary assisted approach was even less successful, giving only modest selectivities.¹⁰⁹ Installation of the auxiliaries at the C-3 position requires a complicated process of installing and then removing the auxiliary from the methylene group at C3. The C-3 auxiliaries employed include halides, acetyl esters, thioethers and phenyl selenides; all of which are at least partially successful in providing the desired equatorial selectivity (Scheme 5).¹¹⁰

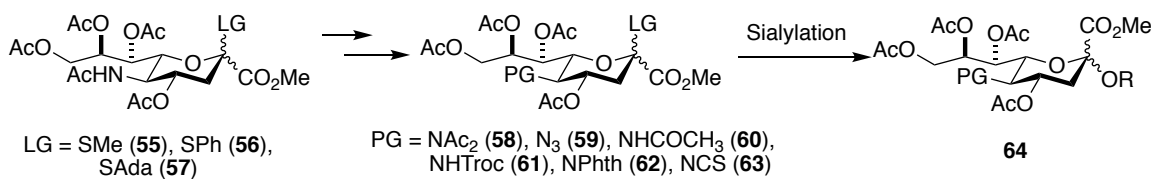


Scheme 5: The general approach of C-3 auxiliary mediated glycosylation

1.7.2 Conversion of the acetamide at C-5 to more electron withdrawing groups

The C5 acetamide of sialic acids can be converted chemically to various protecting groups, which results in changes in the reactivity and stereoselectivity of the glycosylation reactions.¹¹¹ This is important because the stereoselectivity of the

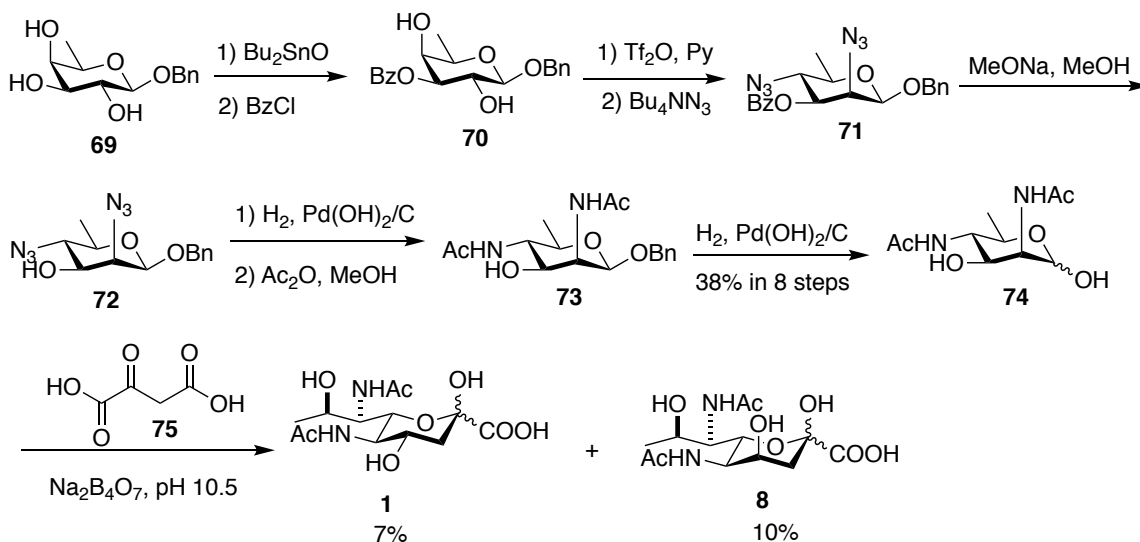
sialylation is improved tremendously by the introduction of various electron withdrawing protecting groups at C-5, such as the NAC_2 ,^{112,113} azide,¹¹⁴⁻¹¹⁷ isothiocyanate,¹¹⁸ trifluoroamide,¹¹⁹⁻¹²² N-Troc,^{123,124} NAcBoc,^{125,126} cbz,¹²⁷ N-Fmoc, N-Alloc and trichloroacetyl^{128,129} groups (Scheme 6). These groups at C-5 have all been reported to increase the equatorial selectivity in glycosylation,¹¹²⁻¹²⁹ especially with primary alcohol acceptors.



Scheme 6: Sialylation approach by various modifications on N-5

1.7.3 Use of cyclic protecting groups

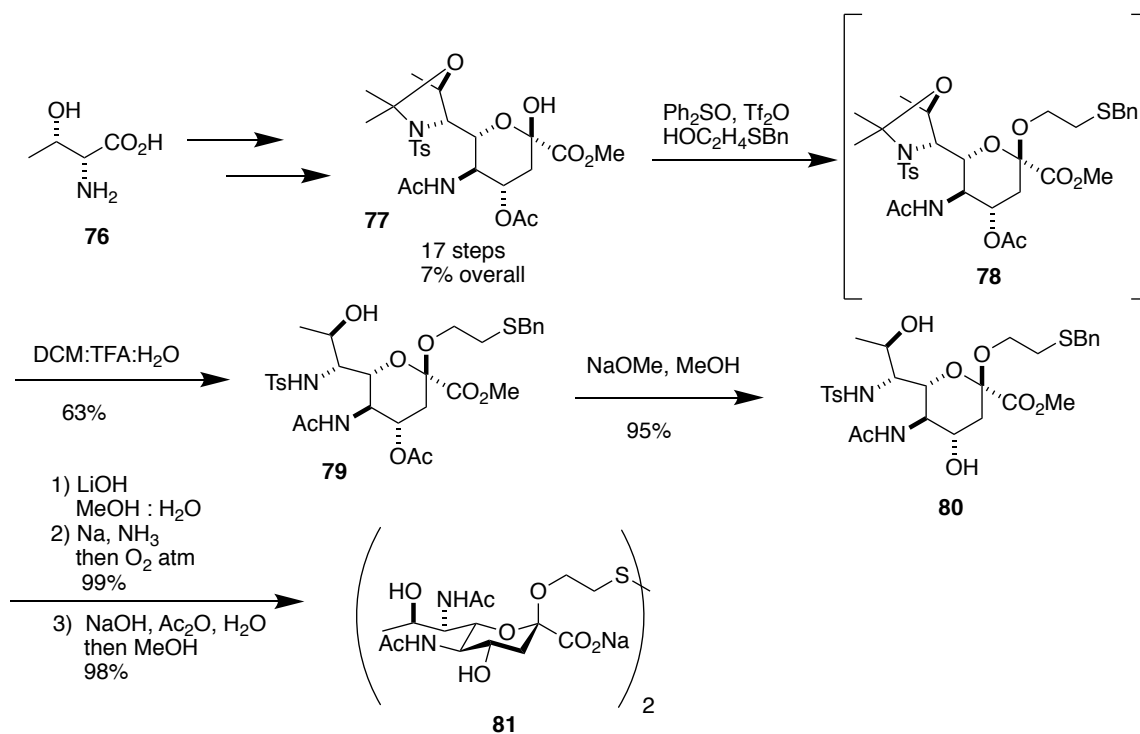
A revolutionary method for the equatorial-selective sialylation introduces a cyclic protecting group on the 4O and 5N positions of sialyl donors. Crich *et al.* synthesized *trans*-fused *N*-acetyl 5*N*,4*O* oxazolidinone protected phenylthio and thioadamantyl sialosides, which gave the excellent glycosylation selectivity and yields under the typical NIS/TfOH in situ activation conditions.^{130,131} The extra acetyl group in the Crich method had the advantage of affording the native C-5 acetamide in very mild condition after glycosylation, in contrast to the simple 5*N*, 4*O*-oxazolidinones developed by Takahashi¹³² and De Meo,¹³³ which required harsher conditions to cleave the 4,5-*O,N*-oxazolidinone ring (Figure 8).



Scheme 7: Tsvetkov's synthesis of legionaminic acid

1.8.2 Seeberger's synthesis of Leg and a glycoside

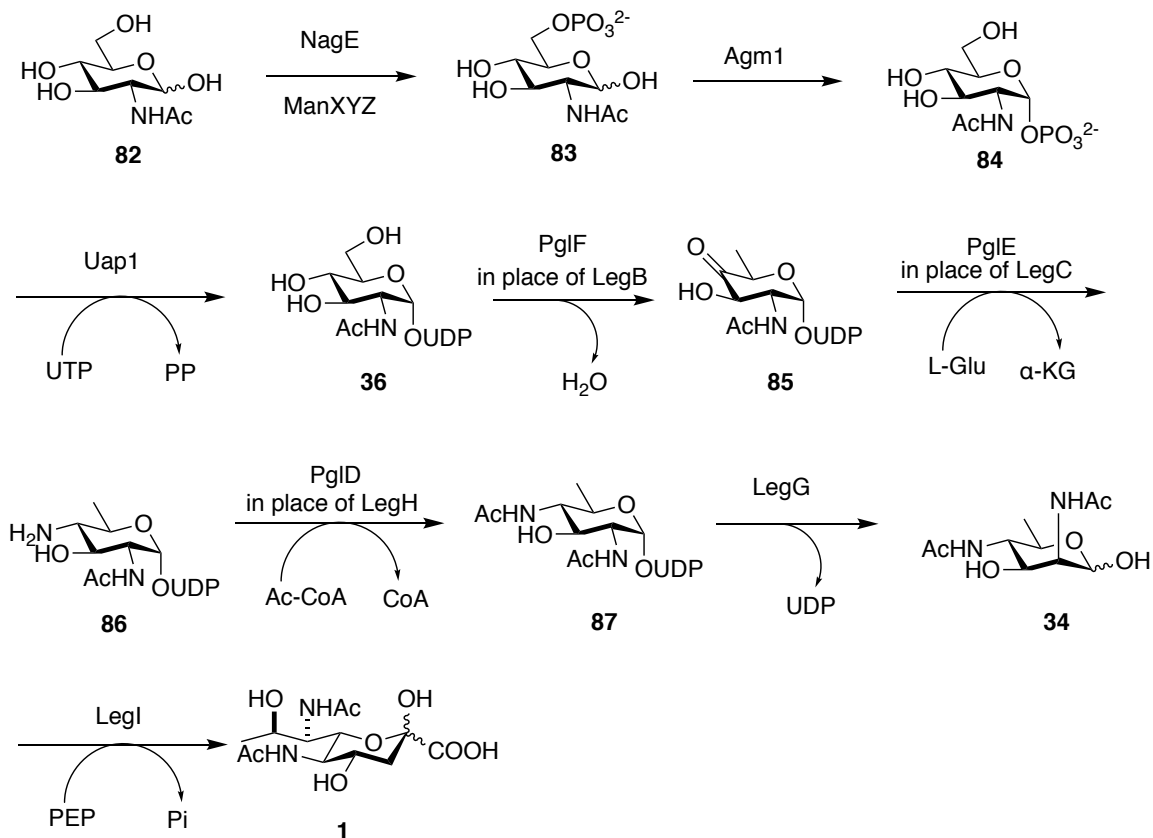
Seeberger *et al.*¹³⁴ performed a *de novo* synthesis of the Leg donor **77** from D-threonine in 17 steps and 7% overall yield. Donor **77** was converted to crude thioether **78** via dehydrative glycosylation. Due to the difficulty associated with purification of **78**, acetal hydrolysis with aq. TFA was performed to give **79** as the axial anomer in 63% yield in two steps. A small amount of the equatorial anomer was obtained in this step but couldn't be purified by silica gel column chromatography and so was not characterized. Deacetylation of compound **79** gave compound **80** in 95% yield. Methyl ester hydrolysis was followed by global deprotection by reductive cleavage of the thioester and the N-sulfonamide groups under Birch reduction conditions. Finally *N*-acetylation of the amine thus obtained gave the acetamide **81**. The overall yield of **81** starting from D-threonine was reported to be 6% (Scheme 8).



Scheme 8: Seeberger's approach to synthesize a leg glycoside

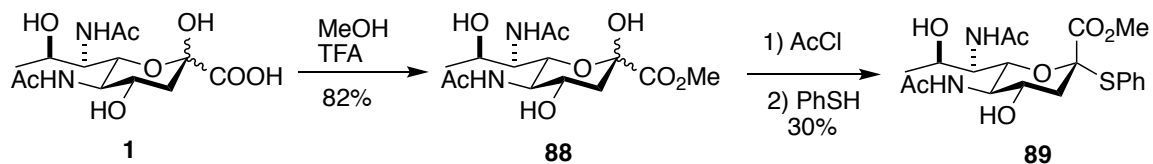
1.8.3 Whitfield's chemoenzymatic synthesis of Leg

Whitfield *et al.*¹³⁵ developed a *de novo* Leg5,7Ac₂ biosynthetic pathway by combining various metabolic modules from three different bacterial sources (*Saccharomyces cerevisiae*, *C. jejuni*, and *L. pneumophila*), taking inspiration from earlier chemoenzymatic work by Tanner *et al.*¹³⁶ Application of the synthetic route in *E. coli* procured a decent amount of legionaminic acid in the culture broth (120 mg L⁻¹) (Scheme 9). Purification of Leg5,7Ac₂ from the culture broth was carried out, which was then activated in the form of CMP-legionaminic acid for biochemical purposes, and a donor in the form of phenyl thioglycoside was synthesized for the chemical glycosylation reactions.



Scheme 9: Whitfield's bioinspired synthesis of legionaminic acid

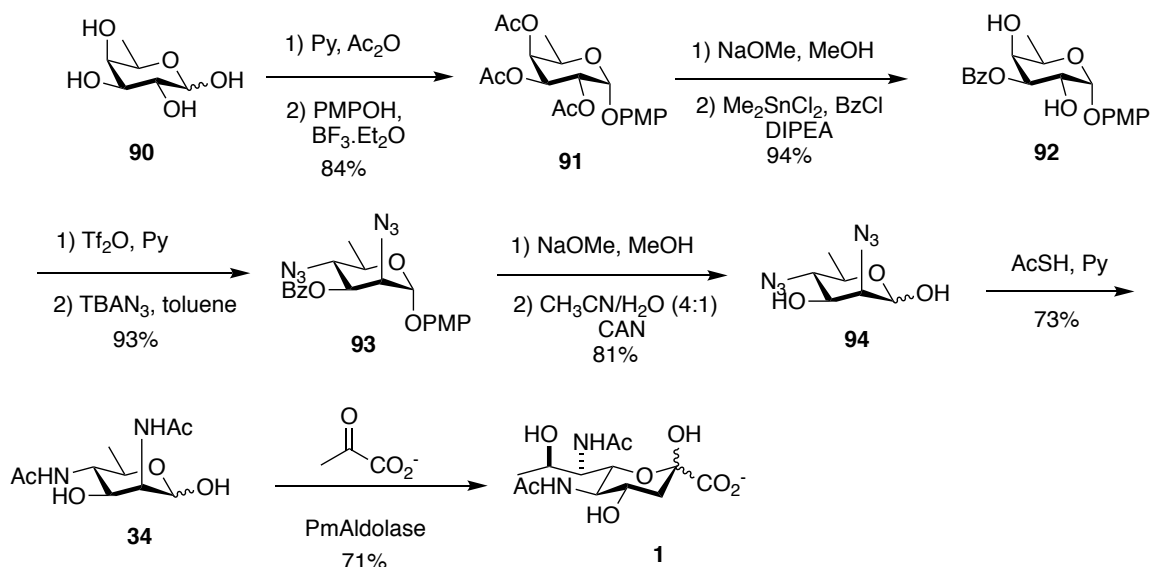
To activate the biosynthesized Leg5,7Ac₂ for chemical glycosylation, it was converted into its methyl ester followed by acetylation and reaction with thiophenol to generate an α -phenylthioglycoside ready for chemical glycosylation. Whitfield *et al.* did not report any glycosylation reaction, but the method provides a donor for possible future chemical glycosylation reactions (Scheme 10).



Scheme 10: Whitfield's further functionalization of Leg to provide a Leg donor

1.8.4 Chen's chemoenzymatic synthesis of Leg and its glycosides

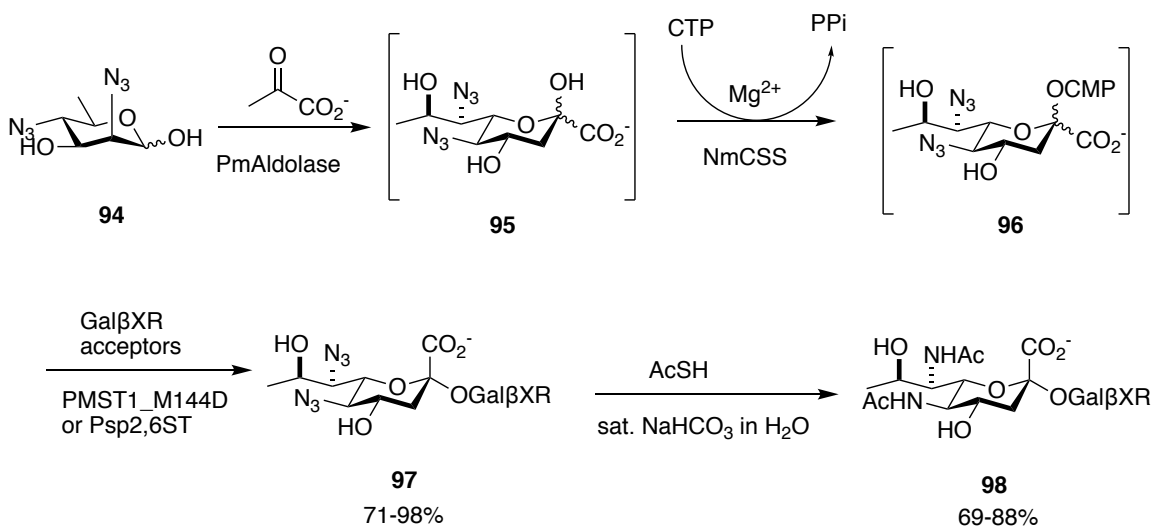
Chen *et al.*¹³⁷ synthesized a library of equatorial (2,3) and equatorial (2,6) linked Leg5,7Ac₂-glycosides from chemically synthesized 2,4-diazido-2,4,6-trideoxymannose (6deoxyMan2,4diN₃) in one-pot multienzyme sialylation method. The six-carbon precursor of Leg5,7Ac₂, 6-deoxy-Man2,4-di-N₃ was synthesized in 9 steps from commercially available D-fucose (Scheme 11).



Scheme 11: Chen's chemoenzymatic approach to legionaminic acid

The diazido precursor **94** was then further functionalized enzymatically to afford a donor, which was then enzymatically glycosylated with three different acceptors, para-nitrophenyl β-glycoside (Gal-β-pNP), thiotolyl β-galactoside (Gal-β-STol) and lactosyl β-propyl chloride (Lac-β-ProCl) to give the equatorial (2,3/6) glycosides depending upon the type of the enzymes employed. The diazido precursor **94** was reacted chemoenzymatically with pyruvate in presence of PmAldolase (obtained from *Pasteurella multocida*) to form the diazido derivative of Leg (**95**, Leg-5,7-di-N₃), which was again reacted with cytidine 5'-triphosphate (CTP) in the presence of *Neisseria*

meningitidis CMP-sialic acid synthetase (NmCSS) to form CMP-Leg-5,7-di-N₃ **96** *in situ*. This was finally converted by a sialyltransferase (PmST1_M144D or Psp2, 6ST) to equatorial (2,3/6) linked Leg-5,7-di-N₃-containing glycosides **97**. When PmST1_M144D was used as the sialyltransferase, equatorial (2,3) linked Leg-5,7-di-N₃-containing glycosides were formed in the yields of 71-98%, while the use of Psp2,6ST as sialyltransferase gave equatorial (2,6) linked Leg-5,7-di-N₃-containing glycosides with the same acceptors in the yields of 73-97%. Among the different strategies used to convert the azido groups of the glycosides to the requisite acetamido groups, thioacetic acid-mediated one-pot conversion in saturated aqueous sodium bicarbonate proved to be the most efficient giving the final Leg-5,7-Ac₂ containing glycosides **98** in 69-88% yields (Scheme 12). The protocol required the use of three different enzymes to obtain the legionaminic acid glycosides.

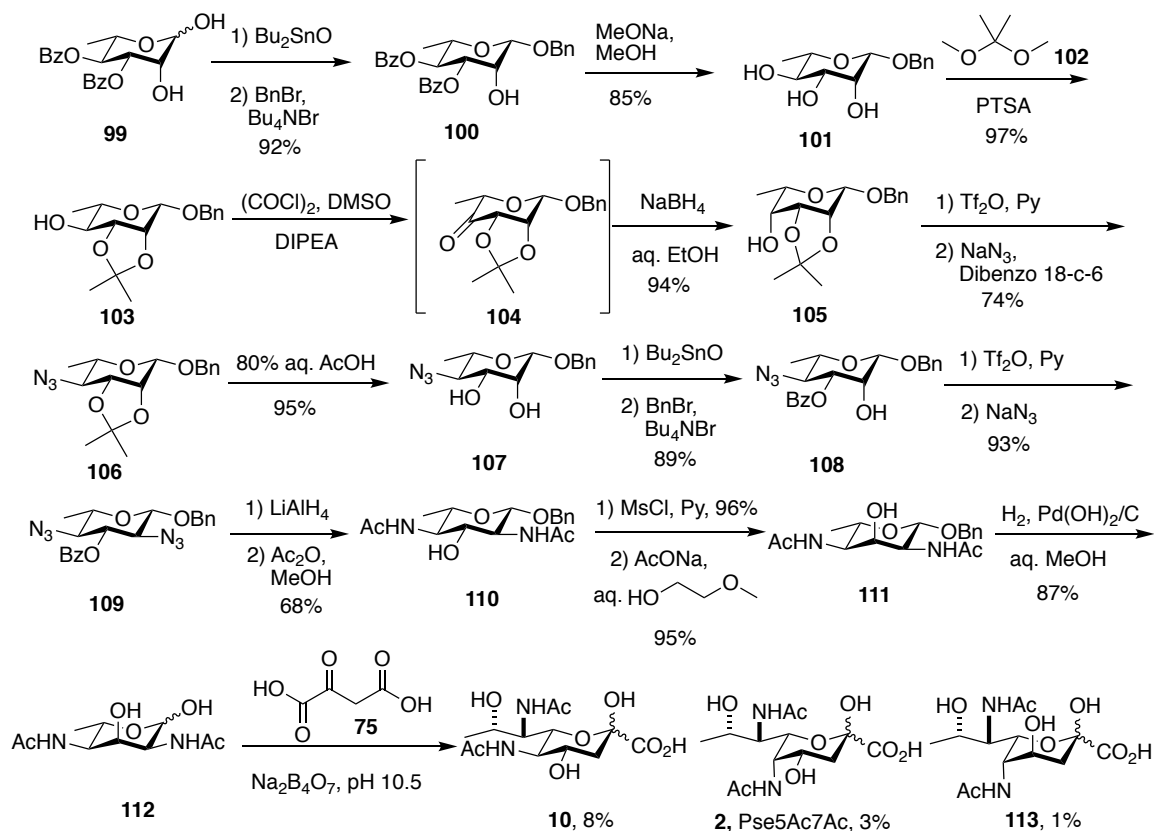


Scheme 12: Chemoenzymatic synthesis of Leg-5,7-di-N₃ followed by glycosylations and deprotections.

1.9 Synthesis of pseudaminic acid and its glycosides

1.9.1 Tsvetkov's synthesis of Pse

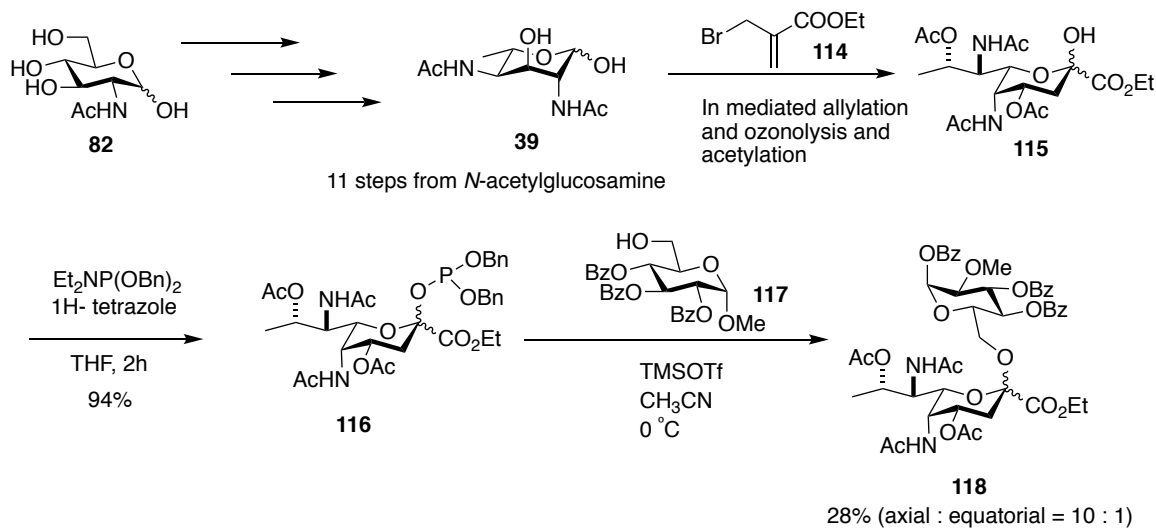
Tsvetkov *et al.*¹³⁸ in 2001 synthesized different classes of 5,7-diamino-3,5,7,9-tetraoxynon-2-ulosonic acids with benzyl β -L-rhamnopyranoside **99** as the precursor of choice. In the final step, the intermediate 2,4-diacetamido-2,4,6-trideoxy-L-allose **113** was reacted with oxalacetic acid in basic conditions to produce pseudaminic acid-diacetate (Pse5Ac7Ac) **2**. The final step was plagued by the formation of a mixture of isomers with the target Pse5Ac7Ac being only a minor product isolated in 3% yield only (Scheme 13). Nevertheless, this method provided the first total synthesis of Pse5Ac7Ac, and enabled confirmation of the definitive structures and configuration of the bacterial nonulosonates paving the way for further work in the field.



Scheme 13: Tsvetkov's approach to synthesize Pse5Ac7Ac

1.9.2 Ito's synthesis of Pse and its glycoside

Ito *et al.*¹³⁹ synthesized pseudaminic acid starting from *N*-acetylglucosamine **82**, which was subjected to deoxygenation and deoxyamination with inversion of configuration to give 6-deoxy-AltDiNAc **39**, the key precursor for the biosynthesis of pseudaminic acid. This precursor was subjected to In-mediated allylation with a bromomethacrylate ester followed by ozonolysis and hydrolysis to give the pseudaminic acid **115**. In order to do a chemical glycosylation reaction, this pseudaminic acid was converted to the dibenzyl phosphite donor **116**. Glycosylation with the primary acceptor **117** in acetonitrile afforded the disaccharide **118**, albeit in only 28% yield, with the undesired axial anomer as the major anomer in the ratio of 10:1 (Scheme 4).

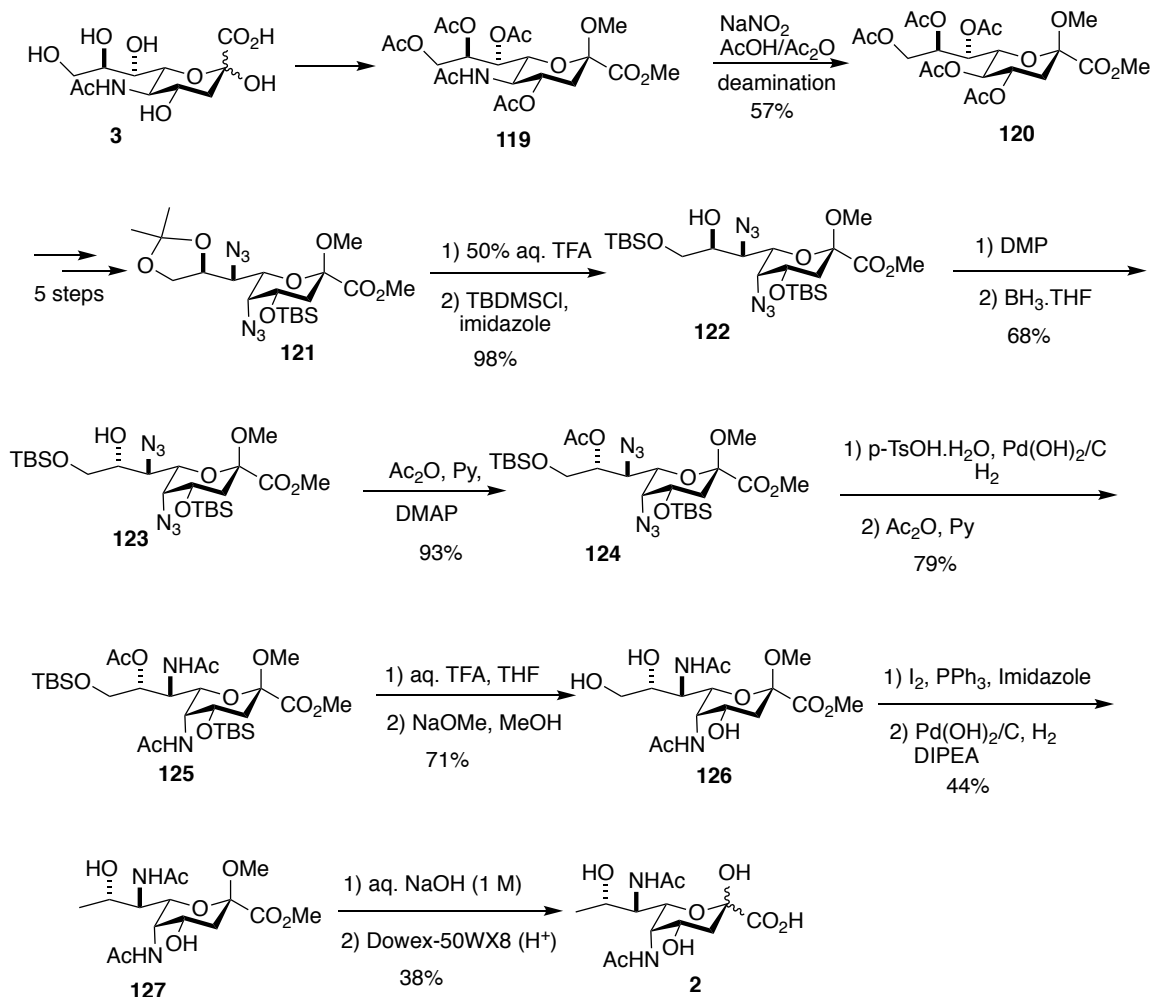


Scheme 14: Ito's synthesis of a pseudaminic acid and its glycoside

1.9.3 Kiefel's synthesis of Pse

Kiefel *et al.* in 2016 reported the synthesis of Pse5Ac7Ac starting from the most common sialic acid, *N*-acetylneuraminic acid (Scheme 15).^{39,140} While the concept of functionalizing the inexpensive *N*-acetylneuraminic acid to get the rare Pse5Ac7Ac is attractive, this method suffered some serious drawbacks. First, the oxidative deamination

of the 5-acetamide using acetic acid as nucleophile resulted in the formation of the penta-acetate **120**, losing the differential functionalization at the 5-position in presence of multiple other acetates. Secondly, the yields toward the end of the synthesis were low making it difficult to scale up the total synthesis. Thirdly, the protocol doesn't provide a Pse donor with which to carry out any chemical glycosylation reactions.

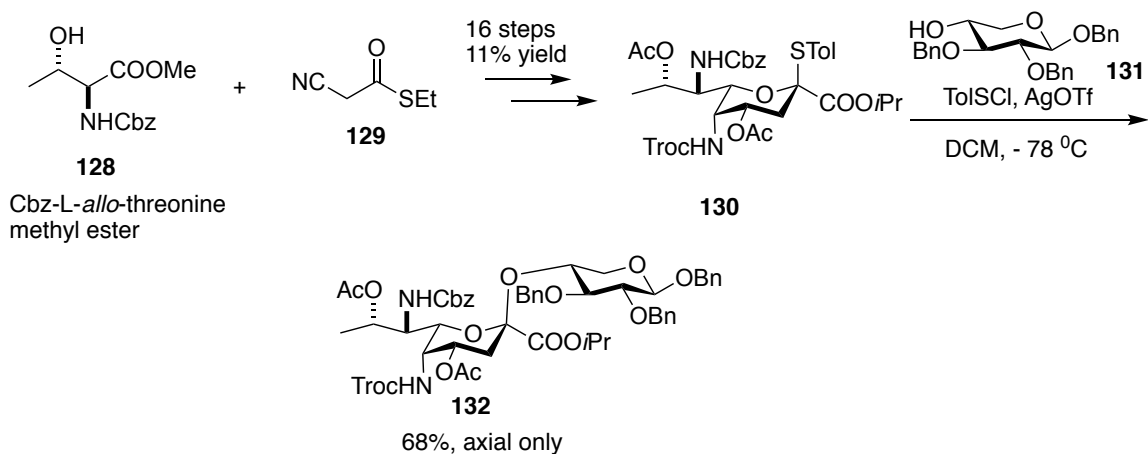


Scheme 15: Kiefel's synthesis of Pse5Ac7Ac starting from Neu5Ac

1.9.4 Li's synthesis of Pse and its glycosides

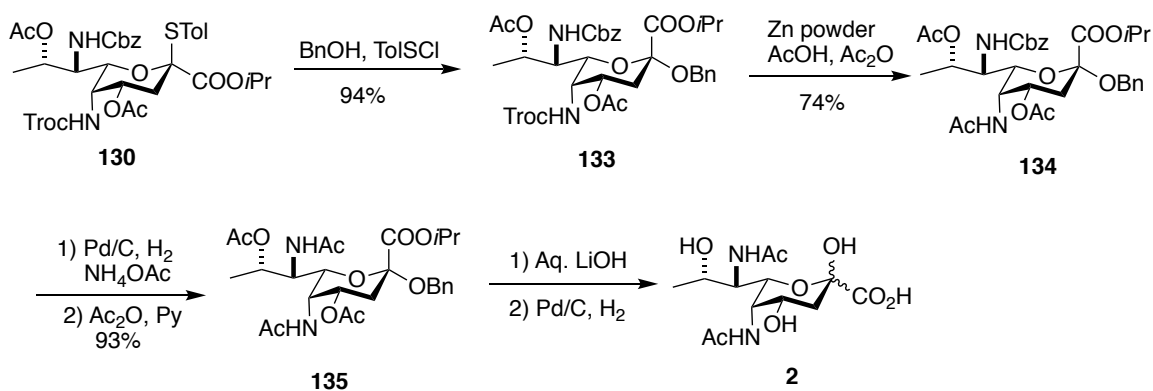
Li *et al.*¹⁴¹ synthesized the Pse donor **130** in 16 steps and 11% overall yields, and performed glycosylation with benzyl alcohol, a monosaccharide, and a disaccharide

acceptors (Scheme 16). In all of the cases, the glycosylated products gave the axial anomers as the major isomers.

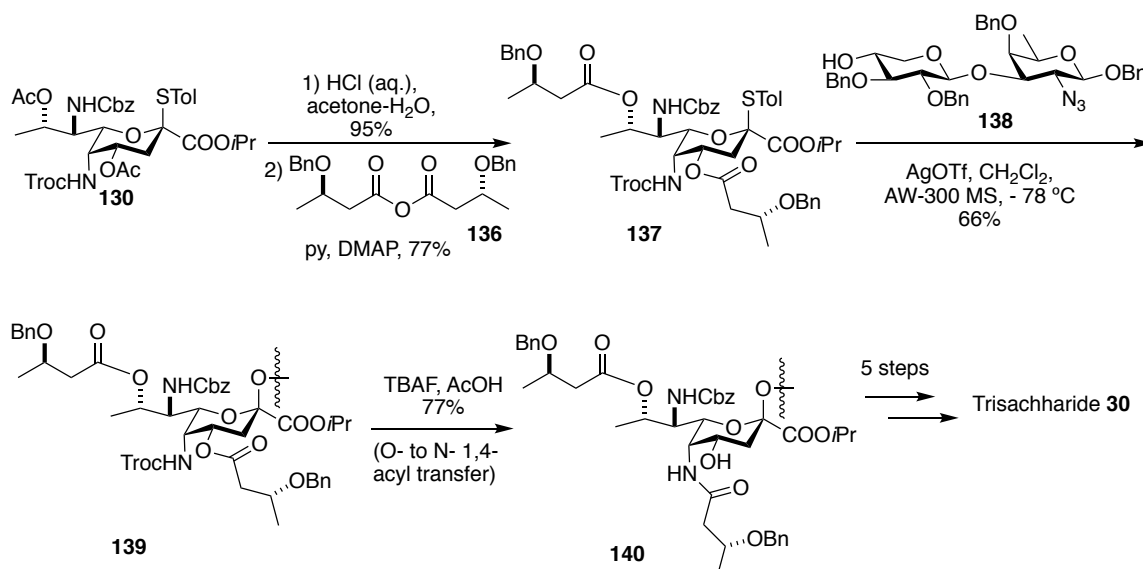


Scheme 16: Li's synthesis of a Pse donor and its glycosylation

Nonetheless, it is significant that Li and coworkers went on to synthesize pseudaminic acid itself (Scheme 17), and also the pilin trisaccharide **30** (Scheme 18).



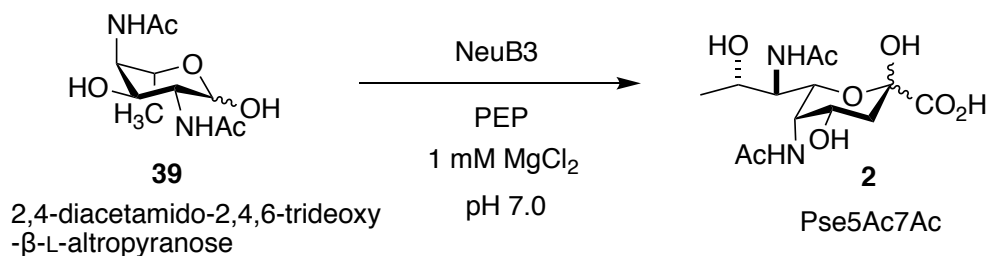
Scheme 17: Glycosylation and deprotection of the Pse donor 130 giving Pse5Ac7Ac



Scheme 18: Glycosylation of the Pse donor 130 giving the trisaccharide 30

1.9.5 Chou's chemoenzymatic synthesis of Pse

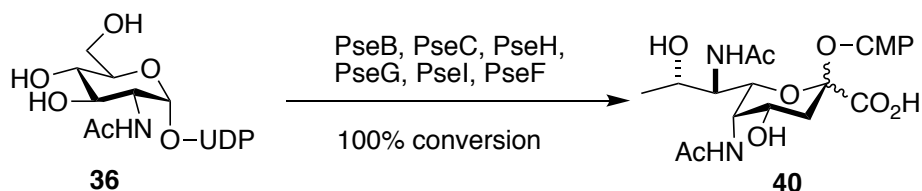
Chou *et al.*¹⁴² in 2005 used a chemoenzymatic approach to synthesize Pse5Ac7Ac from the 2,4-diacetamido-2,4,6-trideoxy- β -L-altropyranose (6-deoxy-AltdiNAc) precursor **39**. The 6-deoxy AltdiNAc precursor **39**, synthesized chemically in 12 known literature steps from L-fucose, was condensed with phosphoenolpyruvate (PEP) in the presence of an enzyme obtained from *Campylobacter jejuni*, pseudaminic acid synthase homolog (NeuB3), to give Pse. It was found that the pseudaminic acid synthase required a divalent ion for catalysis and that the optimal catalysis conditions are at pH 7.0 (Scheme 19).



Scheme 19: Chou's chemoenzymatic synthesis of Pse

1.9.6 Schoenhofen's chemoenzymatic synthesis of Pse

Schoenhofen *et al.*⁹⁷ in 2006 were able to synthesize CMP-Pse from the natural precursor, UDP-GlcNAc. The idea behind this chemoenzymatic approach came from close inspection of the biosynthesis of pseudaminic acid observed in *C. jejuni* and *H. pylori*. Combining all six of the biosynthetic enzymes from *H. pylori* in one-pot, they were able to achieve the first *in vitro* synthesis of CMP-Pse (Scheme 20).



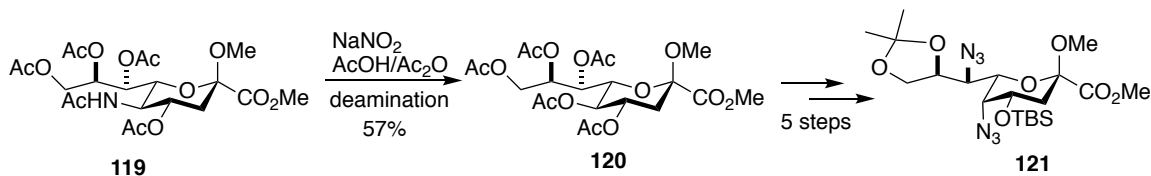
Scheme 20: Schoenhofen's one-pot enzymatic synthesis of CMP-Pse

1.10 Analysis of expected difficulties arising from different configurations

In the last decade or so, NeuAc has become quite cheap and readily available. It can now be purchased in 100 g batches for \$3/g. Therefore, NeuAc can be considered as a starting point for the synthesis of any sialic acid including Pse and Leg. Nevertheless, owing to the several structural and stereochemical differences between NeuAc, Pse, and Leg, methods for the synthesis of NeuAc glycosides don't guarantee success for the Leg and Pse glycosides. In fact, the simple inversion of configuration at the 7-position of NeuAc has led to differences demonstrated in glycosylation reactivity and selectivity of O4, N5-oxazolidinone-type sialic acid donors,¹⁴³ because of the imposed change in side chain conformation.¹⁴⁴ On top of that, because of the differences in configuration between *N*-acetylneuraminic acid and pseudaminic acid at 5-, 7-, and 8-positions and the replacement of the C-O bonds at the 7- and 9-positions by C-N and C-H bonds, the synthesis itself is necessarily challenging.

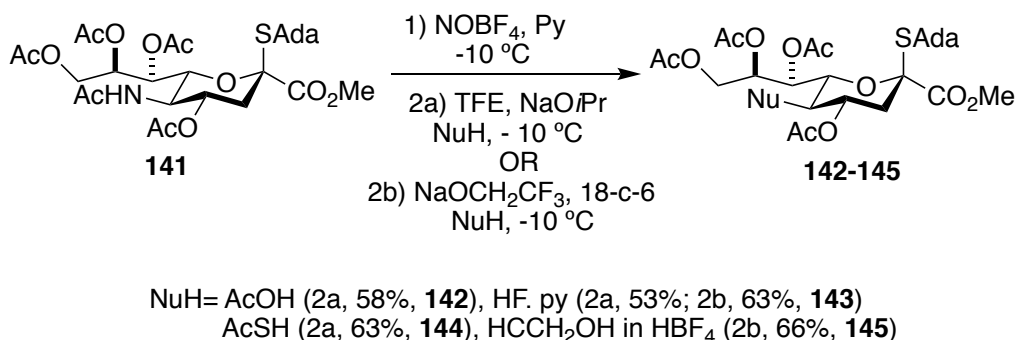
1.10.1 Inversion of configuration at C-5 via oxidative deamination

One of the major hurdles in converting NeuAc to Pse is the inversion of configuration at C-5, the equatorial acetamide of NeuAc needing to be converted to the axial acetamide. The only known way is to perform oxidative deamination of the acetamide to obtain an oxygenated product, and then try invert the configuration. In 2013, Kiefel *et al.*³⁹ achieved the inversion of configuration at C-5 via the oxidative deamination approach using acetic acid as nucleophile *en route* to the synthesis of Pse (Scheme 21).¹⁴⁰ Nevertheless, this approach of using acetic acid as nucleophile resulted in the formation of a penta-acetate requiring subsequent differentiation of ester groups. Further, the protocol demanded heating the acetamido containing substrate to 50 °C, with the strong possibility of losing stereocontrol.



Scheme 21: Kiefel's approach of oxidative deamination *en route* to Pse synthesis

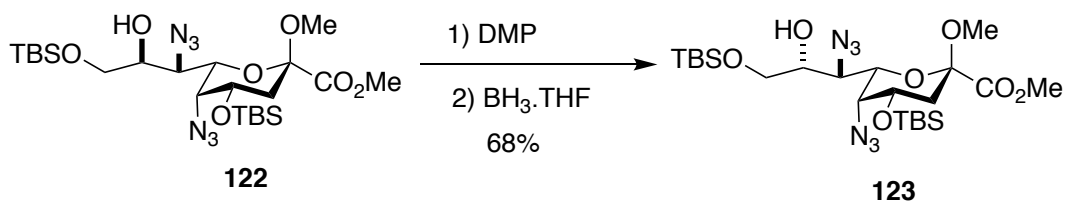
The Crich lab has also been working on the modified Schreiner and Zbiral oxidative deamination of the sialic acids using different nucleophiles under the milder conditions of -10 °C, one of the nucleophile being acetic acid (Scheme 22).¹⁴⁵



Scheme 22: Crich's approach to oxidative deamination

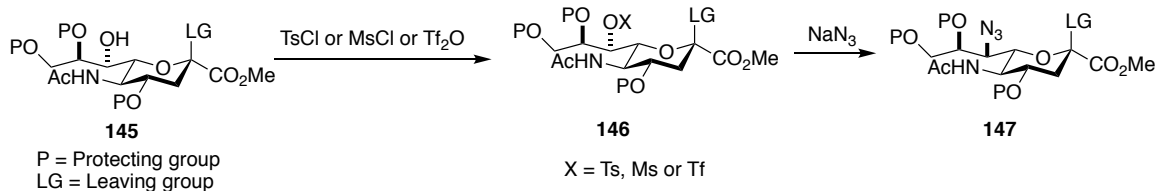
1.10.2 Inversion of configuration at C-8 and C-7

Zbiral *et al.*¹⁴⁶ in 1989 reported the synthesis of 4-*epi* and 8-*epi* *N*-acetylneuraminic acid, utilizing a two-step protocol involving oxidation (RuO_4 in CHCl_3) followed by stereoselective reduction ($\text{BH}_3\cdot\text{NH}_3$ in MeOH) in 80% yield over two steps. Following a similar protocol, Kiefel *et al.* in 2016 inverted the configuration at C-8 using oxidation (Dess-Martin periodine in CH_2Cl_2) followed by stereoselective reduction (1M $\text{BH}_3\cdot\text{THF}$ in THF) in 68% yield in 2 steps (Scheme 23).¹⁴⁰ Fortunately, the major product formed was the desired isomer but the unintended isomer was also formed in 21% yield, and is inconvenient to recycle and tedious to isolate.



Scheme 23: Kiefel's method of oxidation and reduction to invert configuration of C8

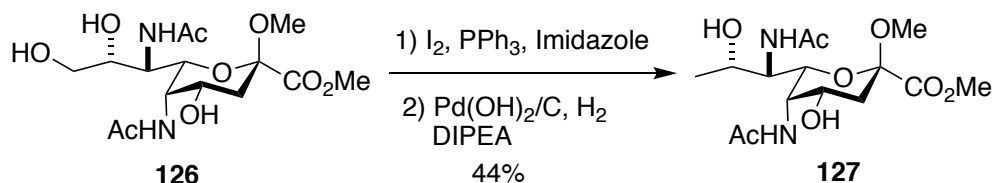
Similarly, inversion of configuration at C-7 with replacement of the C-O bond with a C-N bond is also challenging. The plan would be to convert the hydroxy group to a good leaving group (such as $-\text{OTs}$, $-\text{OMs}$ or $-\text{OTf}$) and then carry out $\text{S}_{\text{N}}2$ substitution with an azide group to install the $-\text{C-N}$ bond with correct configuration (Scheme 24).



Scheme 24: Planned inversion of configuration replacing C-O with C-N at C7

1.10.3 Deoxygenation at C-9

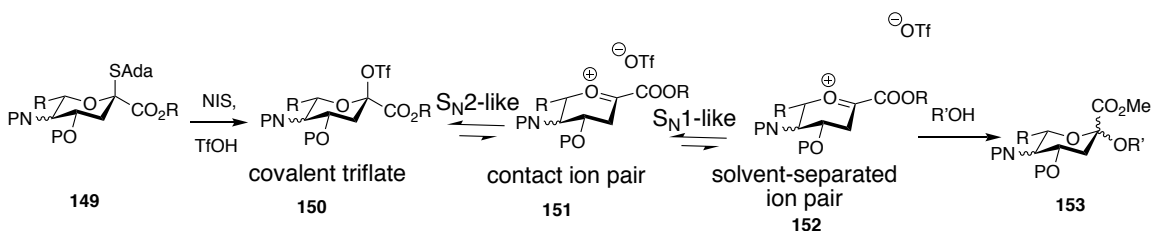
The installation of a C-H bond in place of a C-O bond i.e deoxygenation, at C-9 is another challenging aspect of this total synthesis. Previously, only one case of the deoxygenation at C-9 of a sialic acid had been performed. Kiefel and Payne *et al.* in 2016 achieved this in a two step sequence by first installing an iodo group at C9 and then performing hydrogenolysis to obtain the desired product (Scheme 25).¹⁴⁰ However, this approach yielded only 44% of the desired material in 2 steps.



Scheme 25: Kiefel's approach of C-9 deoxygenation

1.10.4 Effect of C-5 stereochemistry on the glycosylation selectivity

To understand how the C-5 stereochemistry on the sialic acid donor might impact the selectivity on the glycosylation reaction, a general glycosylation mechanism with a common promoter, NIS/TfOH, leading to the final glycosylated product is shown below (Scheme 26).^{147,148}



Scheme 26: General equilibria in the glycosylation of sialic acid with NIS/TfOH as promoter

In general, the equilibrium position and hence the glycosylation selectivity is affected by the ability of the substituents and solvents to shift the equilibria by the differential stabilization of positive charge at the anomeric center. More importantly, electron-withdrawing groups destabilize the positive charge and shift the equilibria to the left toward the contact ion pair (CIP), giving greater equatorial selectivity. While the axial C-O and C-N at C-5 stabilize the positive charge electrostatically better than the equatorial one,^{149,150} leading the equilibria towards solvent-separated ion pair (SSIP), giving more of the axial selectivity. Therefore, sialyl donors with ax C-N bonds are expected to be less selective than the equatorial isomers (similar to that of the relative reactivity and selectivity of gluco and galactopyranosyl donors),¹⁵¹⁻¹⁵⁴ meaning that the equatorial glycosylation selectivity in the pseudaminic acid is expected to be challenging.

1.11 Goals of this thesis

Starting from the commercially available *N*-acetyl neuraminic acid, the donors of rare but important classes of sialic acid, legionaminic acid and pseudaminic acid donors will be synthesized, and their glycosylation reactions with various acceptors will be described. Additionally, the glycosylation selectivity observed will be discussed with the help of the conformational analysis of the exocyclic bond to the side chain. Chapter 2 will describe about the synthesis of a legionaminic acid donor and the stereoselective

synthesis of its equatorial glycosides. Chapter 3 will describe about the synthesis of a azido based *5-epi*-equatorial NeuAc donor related to the pseudaminic acid glycosides and its use in the stereoselective synthesis of *5-epi*-equatorial glycosides. Additionally, the synthesis of a NeuAc donor and its stereoselective synthesis of NeuAc glycosides will be described, and compared with the *5-epi*-equatorial NeuAc in terms of glycosylation reactivity and selectivity. The observations will also be analyzed through side chain conformation of the donors. Finally, in Chapter 4, synthesis of a pseudaminic acid donor and the stereoselective synthesis of its equatorial glycosides will be described, followed by the analysis of its side chain conformation. All the synthesized glycosides will be subjected to straightforward deprotection sequences to give the final fully deprotected glycosides.

CHAPTER 2. STEREOSELECTIVE SYNTHESIS OF THE EQUATORIAL GLYCOSIDES OF LEGIONAMINIC ACID

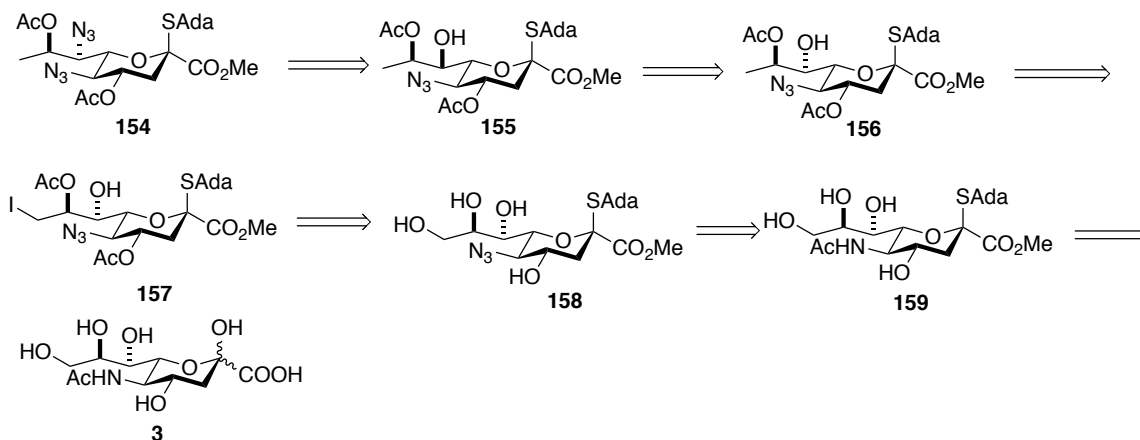
2.1 Background

Legionaminic acid was first identified from the lipopolysaccharide (LPS) of a Gram-negative bacteria, *Legionella pneumophila* in 1994 in the form of the equatorial (2→4) linked homopolymer **26**.³¹ Such a late discovery of legionaminic acid can be attributed to its low abundance in nature and the lack of powerful glycoanalysis tools. With the eventual improvements in analytical tools, legionaminic acid has since been reported in other bacterial glycoconjugates, such as the repeating unit of heteropolymeric LPS, capsular polysaccharides and *O*-linked monosaccharide modifications on the flagellae of numerous Gram-negative pathogens, such as *Campylobacter jejuni*,^{34,35,96} *Cronobacter turicensis*,¹⁵⁵ *Enterobacter cloacae*,¹⁵⁶ and *Acinetobacter baumannii*.¹⁵⁷ Therefore, legionaminic acid glycosides can be considered as candidates for the development of antibacterial vaccines and for use in diagnostic tools.^{134,158} For such studies to be undertaken, greater quantities of legionaminic acid^{135,136} and its glycosides^{40,41} are required. Recent biosynthetic works accessing legionaminic acid and its glycosides are promising but not sufficient, hence practical chemical syntheses are mandatory. Synthetic work by Tsvetkov *et al.*⁴³ and Seeberger *et al.*¹³⁴ provide insufficient amount or unwanted glycosylation selectivity. Synthesis of such an azido based legionaminic acid donor and the study of its glycosylation reactions is the focus of the work described in this chapter.

2.2 Results and discussion

2.2.1 Retrosynthetic analysis of the synthesis of a legionaminic acid donor

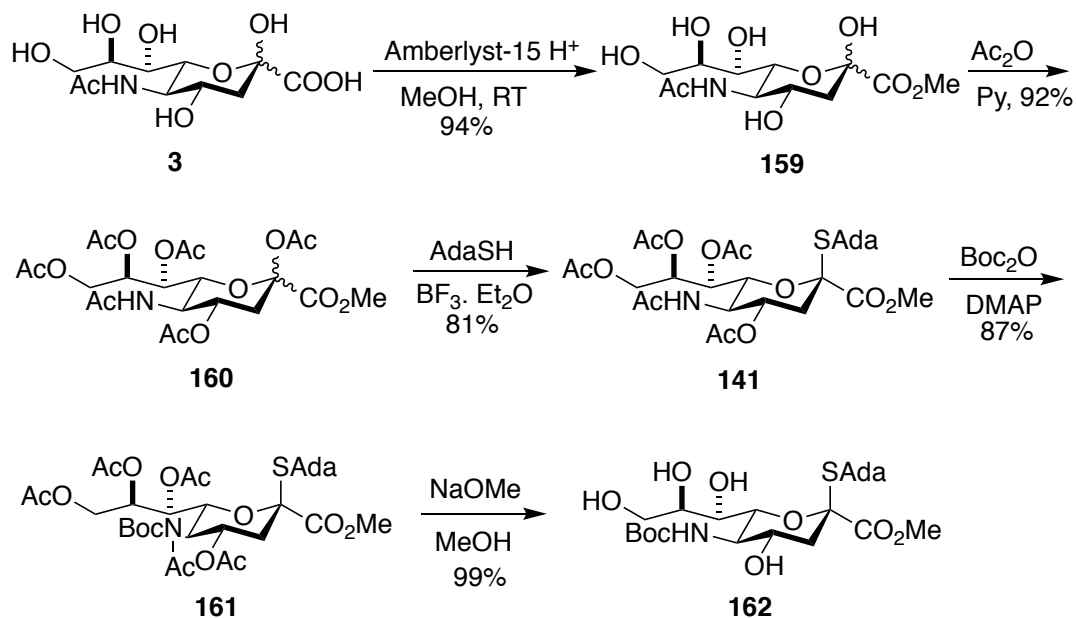
N-acetylneuraminic acid is the starting material for the synthesis of the legionaminic acid donor **154**, owing to its low cost commercial availability. The thioadamantanyl group is the preferred leaving group in the synthesis of the thioglycoside **159**, as it is relatively easy to activate under the glycosylation conditions at $-78\text{ }^{\circ}\text{C}$. The use of the electron-withdrawing azide group as in the case of **158** as amine protection is a logical one, as similar electron-withdrawing groups such as the 4,5-acetyloxazolidinone,^{130,131} oxazolidinone,^{132,133} isothiocyanate¹¹⁸ moieties furnished equatorial glycosylation selectivity. The azide group can also be readily installed and deprotected, making it one of the best amine protecting groups. The C-5 acetamido group can be converted to the azido group in a sequence of reactions consisting of Boc installation, Zemplen deacetylation, removal of Boc group and then use of Stick's reagent to the synthesized amine intermediate. This sequence of reactions requires a selective protection of 4- and 8-hydroxy groups to be performed in presence of 7-hydroxy group such as in **157**. The 7-hydroxy group has been found to be the most hindered hydroxy group in *N*-acetylneuraminic acid,¹⁶⁰ so this reaction is quite feasible. Finally inversion of configuration at C-7 followed by triflation and azido installation in $\text{S}_{\text{N}}2$ fashion was expected to furnish the desired legionaminic acid donor such as **154** (Scheme 27).



Scheme 27: Retrosynthesis of a planned legionaminic acid donor 154

2.2.2 Synthesis of the 5-*N*-Boc protected thioadamantanyl sialoside 162

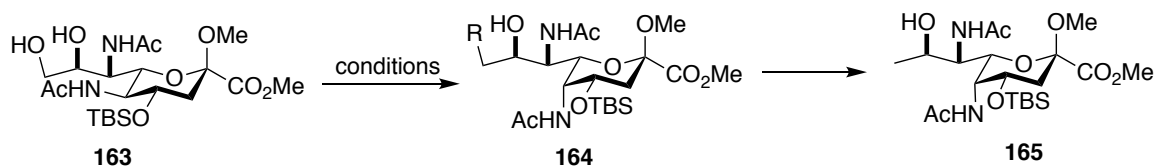
Synthesis of the Leg donor started from *N*-acetylneuraminic acid, which was converted to the *N*-Boc adamantanyl thioglycoside **162** in five known straightforward literature steps.^{131,159} The first step was esterification of NeuAc using Amberlyst-15 H⁺ ion-exchange resin to obtain **159**, which was then acetylated to give **160**.¹⁶¹ Thioglycosylation of **160** using 1-adamantanethiol in the presence of boron trifluoride etherate gave **141**,¹³¹ which was the common intermediate for use in the synthesis of all of the sialic acid donors. Boc installation on the amine group of **141** gave **161**,¹³¹ which was followed by Zemplen deacetylation to give **162** (Scheme 28). All of these steps proceeded with excellent yields.



Scheme 28: Synthesis of the 5-*N*-Boc protected thioglycoside 162

2.2.3 Selective functionalization of the 9-hydroxy group

Selective functionalization of the primary hydroxyl group at C9 of sialic acid in the presence of other secondary hydroxyl groups is not a new topic. Kiefel *et al.* has attempted installing few groups at the C9 position, either by replacing or protecting the hydroxy groups of **163** in attempts to synthesize **165** (Scheme 29).³⁹

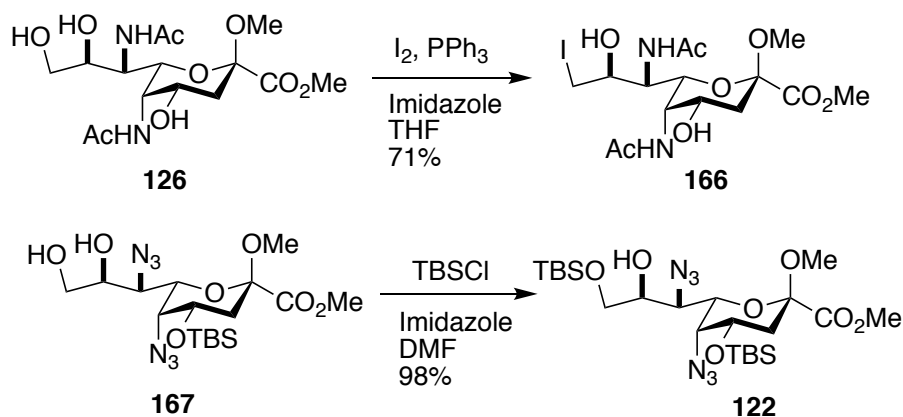


Scheme 29: Overview of Kiefel's attempt to selectively functionalize C9-hydroxy

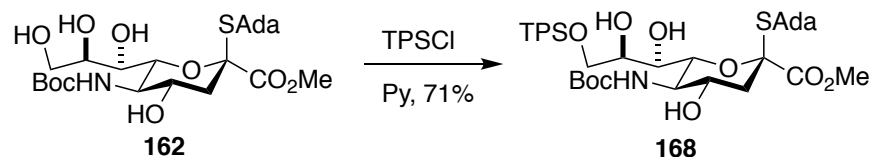
Table 3: Conditions attempted by the Kiefel group to form 164

R =	Conditions	Product
1. I	I ₂ , PPh ₃ , imidazole, THF, Reflux, 2 h	N.R.
2. Br	CBr ₄ , PPh ₃ , toluene/MeCN, RT, 12 h	N.R.
3. OTs	TsCl, pyridine	N.R.
4. H	2-nitrobenzenesulfonylhydrazide, PPh ₃ , DIAD, THF	N.R.

Unsuccessful attempts by Kiefel *et al.* in functionalizing C9 hydroxy group (Table 3) suggested some activation issues. Particularly, the lack of reaction with tosyl chloride was surprising. Finally, a modified Appel reaction to install an iodo group on **126** gave **166**. It was also able to install a TBDMS protecting group selectively in **167** to give **122** were applied at C9 in attempts to access pseudaminic acid (Scheme 30).³⁹

**Scheme 30: Kiefel's successful functionalization of the C9-OH to give 166 and 122**

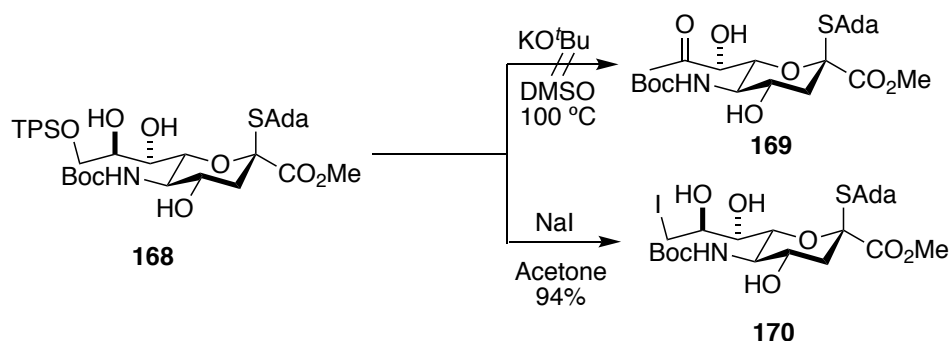
In the light of Kiefel's work, a novel approach to selective sulfonylation of the C9 primary hydroxyl group of **162** with 2,4,6-trisopropylbenzenesulfonyl chloride (TPSCI) in pyridine was attempted and gave sulfonate ester **168** in 71% yield (Scheme 31).¹⁶²



Scheme 31: Selective trisylation of the C9 hydroxy group to give 168

2.2.4 Synthesis of a 9-iodo dibenzoylated sialoside

Functionalization of the trisyl (OTPS) group in compound **168** was attempted next to access the 9-deoxy derivative of the sialic acid thioglycoside. Heating **168** in presence of potassium *tert*-butoxide as base in dry DMSO¹⁶³ at 100 °C didn't proceed to give the expected product **169** via the formation of an enol, probably due to the hindered nature of the tertiary proton at C-8. However, when **168** was heated in acetone with excess sodium iodide the iodo product **170** was obtained in excellent yield (Scheme 32).¹⁶²



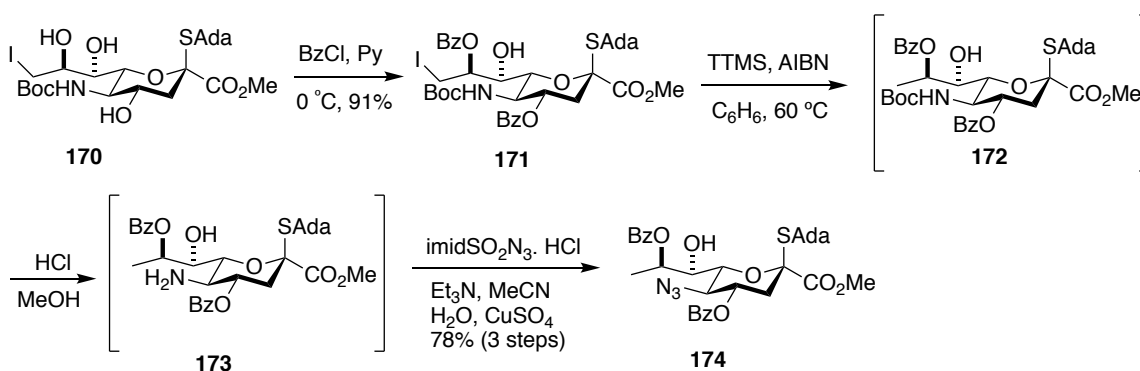
Scheme 32: Attempts at the synthesis of 9-deoxy derivative of 168

2.2.5 Deiodination and synthesis of 5-azido sialoside.

Selective benzylation of **170** with benzoyl chloride in pyridine at 0 °C afforded the 4,8-di-*O*-benzoate **171** in 91% yield, which followed the established pattern of reactivity in the *N*-acetylneuraminic acid series.¹⁶⁰ Selective acetylation was the preferred choice, but was less selective and resulted in formation of a substantial amount of triacetate product. Thereafter, radical reaction conditions with tris(trimethylsilyl)silane¹⁶⁴

and initiation by azoisobutyronitrile in benzene at 60 °C gave the dehydroiodinated product **172**. Partial cleavage of the carbamate by the silyl iodide generated during the reaction was seen. Therefore, the crude product was directly carried forward into the next step of removal of Boc group to obtain the hydrochloride salt of the amine **173** using hydrogen chloride in ether. The crude amine **173** was converted to the corresponding azide **174** in 78% overall yield from **171** with the help of Stick's reagent^{165,166} and triethylamine in the presence of catalytic copper sulfate in aqueous acetonitrile (Scheme 33). Traditional bases for the azidation reaction, such as sodium carbonate or potassium carbonate were not used in place of triethylamine to suppress competing debenzoylation, while the more common aqueous methanol was not used in place of aqueous acetonitrile due to the poor solubility of **173** in methanol.

Hydrogenolytic methods to obtain **172**, both in batch and flow conditions, were also successful for the hydrodeiodination step, but it was not possible to fully suppress the concomitant hydrogenolysis of the thioglycoside.

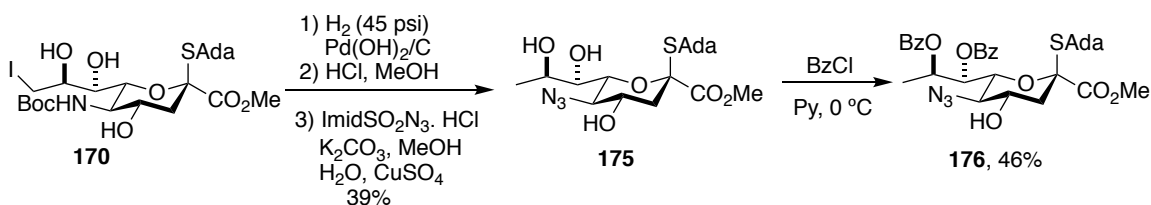


Scheme 33: Deiodination of 170 and synthesis of 5-azido thioglycoside 174

2.2.6 Selective formation of a 7,8-di-O-benzoate

One of the alternative methods attempted to access **174** was the reversal of the deiodination and regioselective benzylation steps. For this, hydrogenolysis of **170** over

palladium hydroxide on charcoal in methanol was carried out to give a deiodo derivative followed by acid mediated deprotection of the Boc group, and then reaction with Stick's reagent in the presence of catalytic copper sulfate and potassium carbonate in aqueous methanol to give the triol **175** in 39% overall yield for the three steps from **170** (Scheme 34). However, treatment of **175** with benzoyl chloride in pyridine at 0 °C did not afford the anticipated 4,8-di-*O*-benzoate **174**; rather it gave 7,8-di-*O*-benzoate **176** in 46% yield (Scheme 34). Typically, in neuraminic acid derivatives, as has been seen in the conversion of **170** to **171**, functionalization preferentially takes place at 4-OH in the presence of 7-OH.¹⁶⁰ The selectivity reversal in the case of **176** suggests that the conversion of **170** to **171** depends on the presence of a functional group at the 9-position, perhaps due to steric buttressing, and is reversed for **176** due to 9-position being unfunctionalized. Alternatively, the difference in regioselectivity may be due to the differing functionalities on nitrogens.

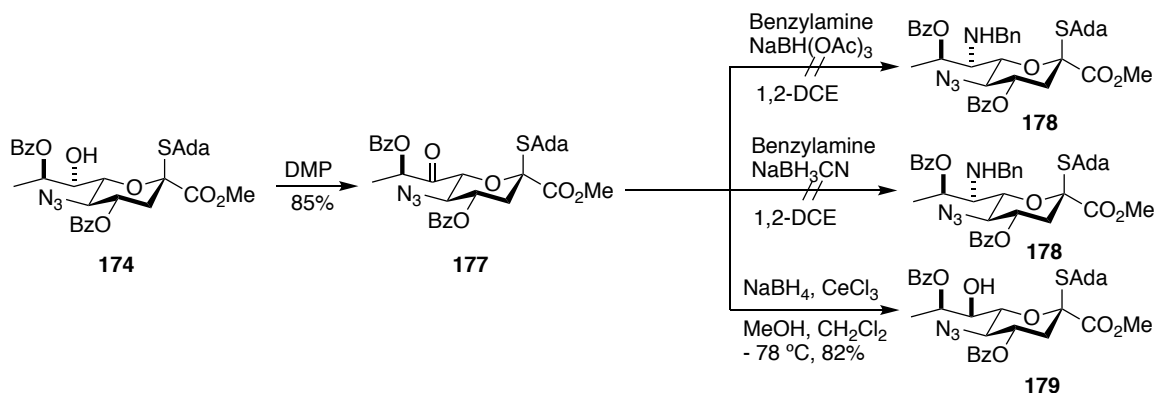


Scheme 34: Selectivity reversal of 170 to give the 7,8-di-*O*-benzoate 176

2.2.7 Inversion of configuration of the 7-hydroxy group

Alcohol **174** was converted to ketone **177** with Dess-Martin periodinane oxidation¹⁶⁷ in 85% yield. Attempted replacement¹⁶⁷ of the C7-hydroxy of **174** with an amine functionality in a single step reductive amination of **177** to obtain **178** involved reacting **177** with benzylamine with either sodium triacetoxyborohydride¹⁶⁸ or sodium cyanoborohydride.¹⁶⁹ However, the expected product **178** was not obtained. Therefore,

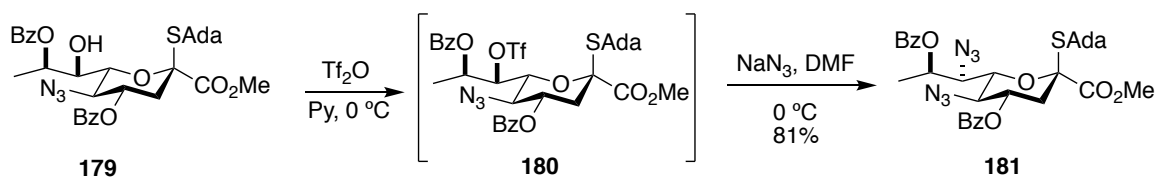
the more straightforward but longer route of double stereoinversion at C-7 was performed. Thus, consistent with the precedent in the *N*-acetylneuraminic acid series,^{143,170} Luche reduction¹⁷¹ of **177** in methanolic dichloromethane at -78 °C afforded a 85:15 mixture of epimeric alcohols from which **179** was isolated in 82% yield, giving the first stereoinverted product in the sequence (Scheme 35).



Scheme 35: Inversion of configuration at C7 of 174

2.2.8 Synthesis of a 5,7-diazido Leg donor

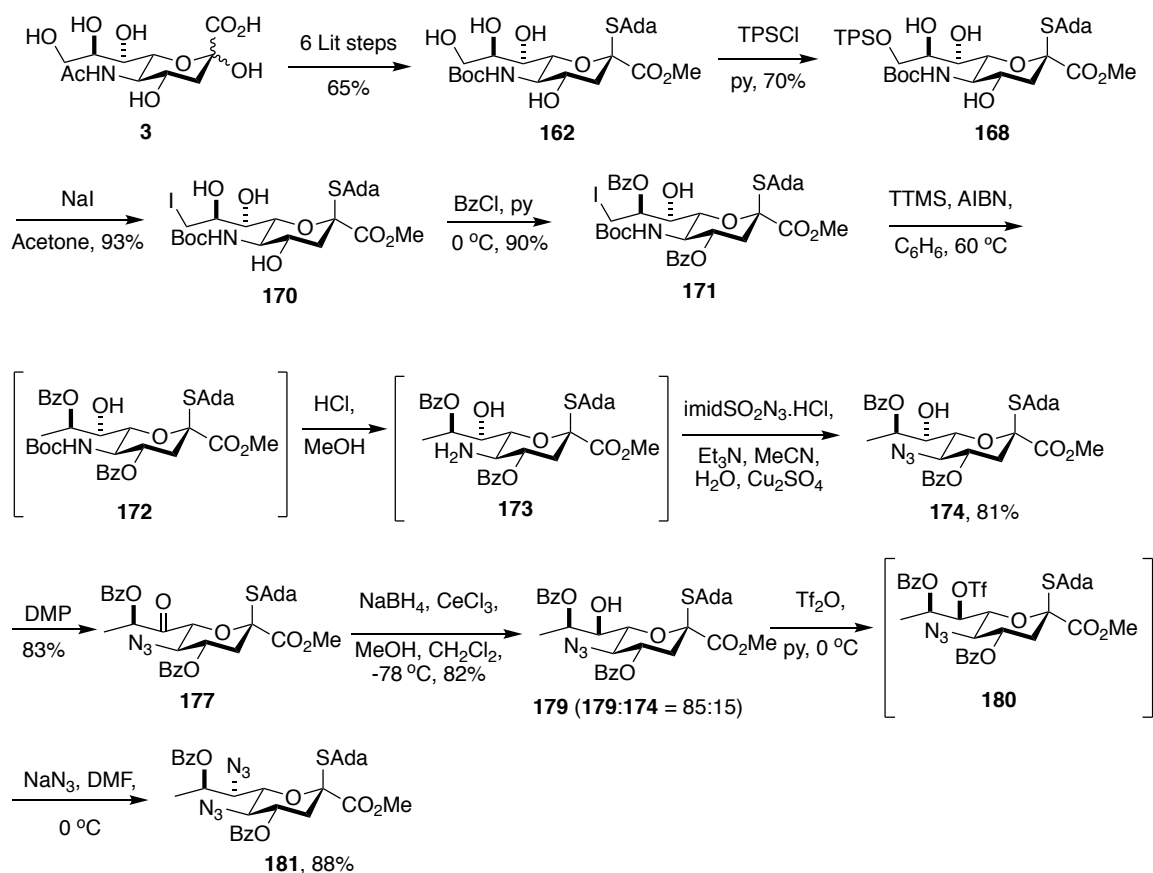
In order to access an amine functionality at C7, a second stereoinversion was performed on **179**. Thus, triflation of **179** with triflic anhydride in pyridine in dichloromethane at 0 °C afforded **180**, which was treated with excess sodium azide in DMF at 0 °C to give the desired legionaminic acid donor **181** in 81% yield (Scheme 36).



Scheme 36: Synthesis of the 5,7-diazido Leg donor 181 from 179

In summary, a legionaminic acid donor was synthesized in 15 straightforward steps and in 17% overall yield from the commercially available *N*-acetylneuraminic acid, as summarized below in Scheme 37. This synthesis is a significant improvement over that

described by the Seeberger laboratory from D-threonine¹³⁴ achieved in 17 steps and 7% overall yield.

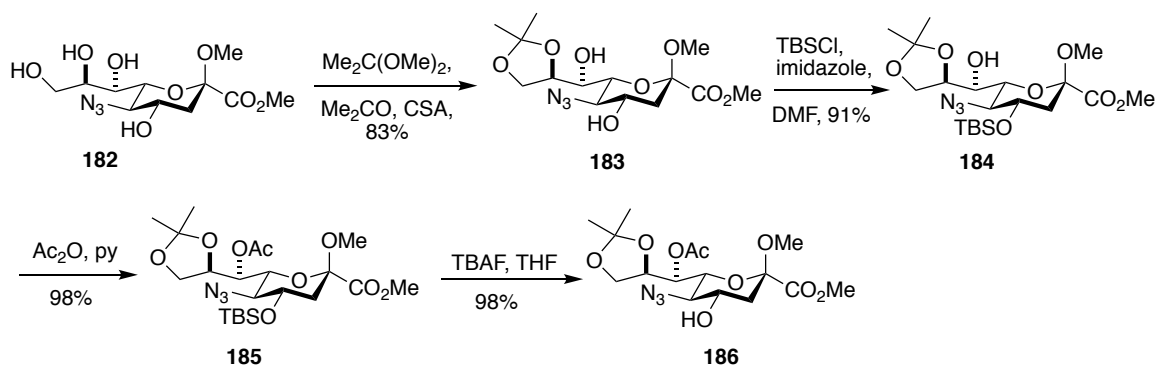


Scheme 37: Total synthesis of the Leg donor 181 from NeuAc, 3

2.2.9 Synthesis of a sialic acid acceptor

With the eventual synthesis of polylegionaminic acid in mind, a sialic acid based acceptor carrying a free hydroxyl group at the 4-position was synthesized to perform glycosylation reaction with the new legionaminic acid donor **181**. The triol **182** having an azido group, readily accessed from NeuAc by known methods,¹⁵⁹ was converted to 8,9-*O*-acetonide **183** in 83% yield. This was followed by selective silylation at the 4-position to give mono silyl ether **184** in 91% yield. Acetylation of the remaining hydroxyl group gave **185** in 98% yield, and TBAF mediated cleavage of the silyl ether afforded the

acceptor **186** in 98% yield, ready for the glycosylation with the legionaminic acid donor **181**.



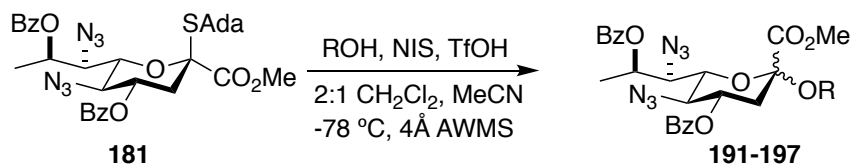
Scheme 38: Synthesis of the sialic acid acceptor 186

2.2.10 Glycosylation study with the Leg donor

Glycosylation reactions of suitable acceptors with the legionaminic acid donor **181** was performed at $-78\text{ }^{\circ}\text{C}$ in 1:2 acetonitrile/dichloromethane in the presence of acid-washed 4Å molecular sieves, with the activation being achieved by the combination of *N*-iodosuccinimide and trifluoromethanesulfonic acid. All reactions were quenched by addition of triethylamine at $-78\text{ }^{\circ}\text{C}$ (Scheme 39).

All the glycosylation reactions afforded equatorial glycosides in good to excellent yield and selectivity. As expected, the best selectivity was observed with the reactive primary acceptor benzyl alcohol followed by the primary carbohydrate-based acceptor **187**. The other two secondary carbohydrate-based acceptors **188** and **189** also gave good selectivity. The galactose based 3,4-diol **189** gave three glycosylated products, two of them being equatorial and axial 2→3 linked products in comparable selectivity, and the third being an equatorial 2→4 linked product. Glycosylation of a relatively less reactive tertiary alcohol **190**, 1-adamantanol, also gave satisfactory selectivity, in fact better than with the secondary alcohols. The glycosylation selectivity observed with the sialic acid

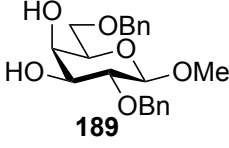
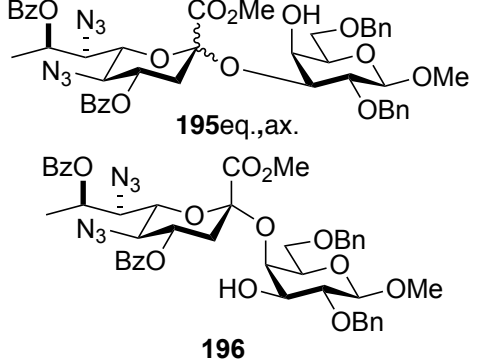
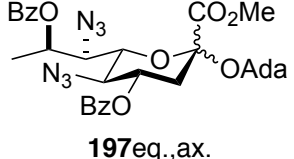
acceptor **186** was also comparable with the other secondary acceptors (Table 4). The glycosylation experiments showed that the azido based legionaminic acid donor **181** is highly beneficial in imparting equatorial selectivity.



Scheme 39: Glycosylation with Leg donor 181

Table 4: Glycosylation reactions of various acceptors with 181

Entry	Acceptor	Product	% Yield, eq.:ax. ratio	³ J _{CH} (Hz)
1	BnOH		96%, eq.-only	191 : 7.2 Hz
2			87%, 6.7:1	192 eq.: 6.4 Hz
3			82%, 4.4:1	193 eq.: 6.9 Hz
4			75%, ^a 4.5:1	194 eq.: 7.2 Hz 194 ax.: 0 Hz

5	 <p style="text-align: center;">189</p>	 <p style="text-align: center;">195eq.,ax.</p> <p style="text-align: center;">196</p>	88%, ^b 4.7:1: 0.9	195eq: 7.0 Hz 196eq: 6.3
6	1-adamantanol, 190	 <p style="text-align: center;">197eq.,ax.</p>	73%, 4.2:1	197eq: 5.7 Hz

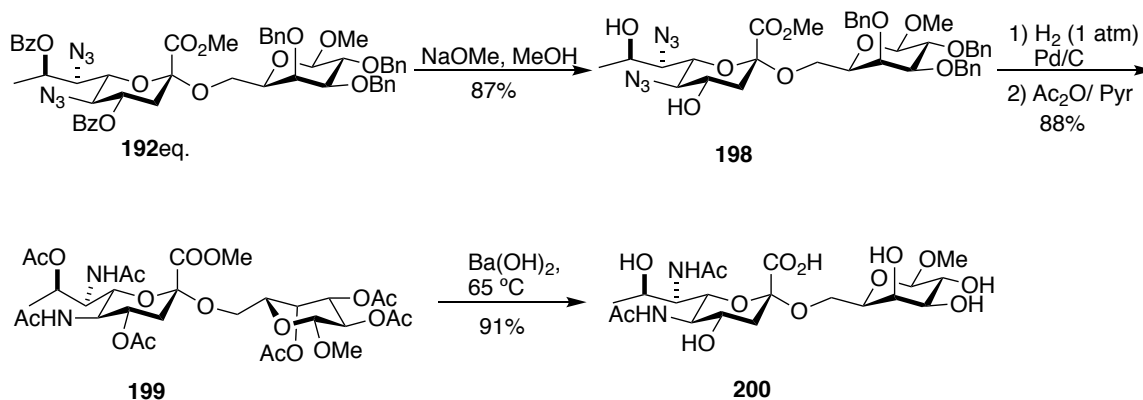
a) Glycosylation gave an 84% yield of an inseparable 4.5:1 eq.:ax. mixture of the disaccharides, which was saponified to give the products **194eq.** and **194ax**; b) In order to determine the regioselectivity of the glycosylation with **190**, a chemical correlation was done as described in section 2.2.14.

The anomeric configuration of the synthesized glycosides was assigned based on the heteronuclear $^3J_{C1,H3ax}$ coupling constant method as discussed below in section 2.2.14.¹⁷²⁻¹⁷⁵ A rationale for the loss of some selectivity in the glycosylation of Leg donor, as compared to a 5-azido NeuAc donor synthesized in Chapter 3, invokes the 9-deoxy nature of the side chain, which makes it less disarming, hence affording greater oxocarbenium-like character in the transition state. Additionally, the electron-withdrawing ability of the azido group in the 7-deoxy-7-azido substitution can also play a role in the change of selectivity.¹⁷⁶

2.2.11 Deprotection of regioisomeric equatorial-(2→6) galactosyl disaccharide

The equatorial 2→6 linked dibenzoyl disaccharide **192** obtained as the glycosylated product was subjected to a four-step deprotection sequence to afford the final fully deprotected disaccharide **200**. Compound **192** was converted to the corresponding diol with sodium methoxide in methanol to give **198** and then by

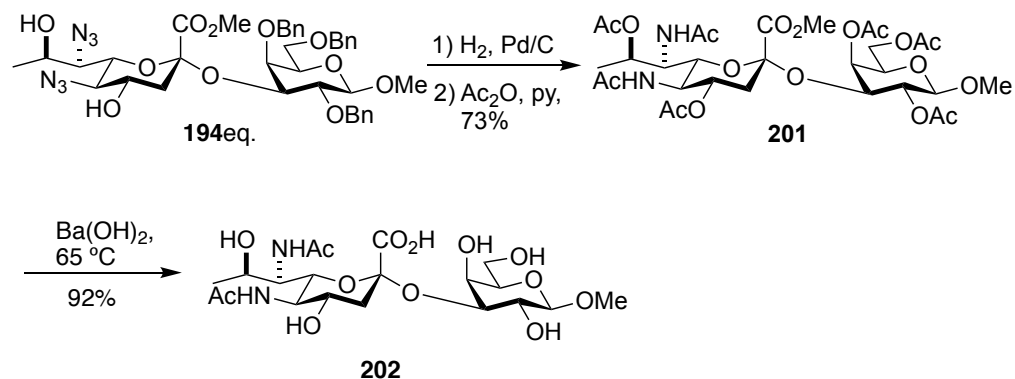
hydrogenation and peracetylation to give **199**. The final global hydrolysis of all esters was carried out by hot aqueous barium hydroxide to give *N,N*-diacetyllegionaminic acid glycoside **200** in good overall yield (Scheme 40). Initial removal of the two benzoate esters was selected before the hydrogenolysis of the azide groups to avoid possible O→N benzoate migration.



Scheme 40: Deprotection of equatorial 2→6 galactosyl disaccharide 192 to give 200

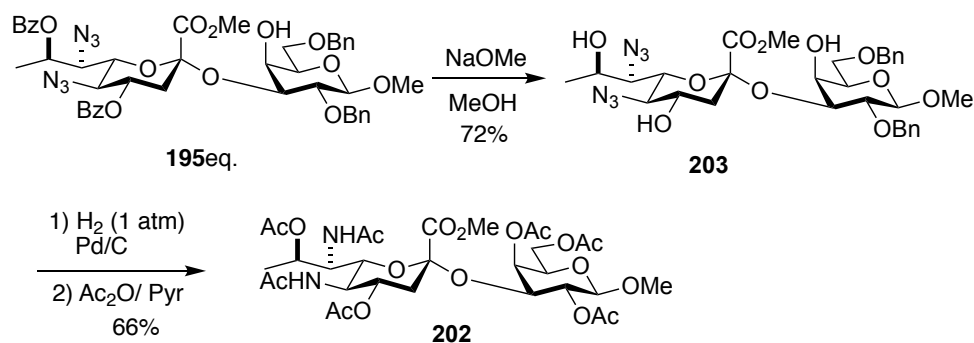
2.2.12 Deprotection of regioisomeric equatorial (2→3) galactosyl disachharide

The equatorial (2→3) linked disaccharide **194** from which the benzoate esters, already deprotected to facilitate separation of the anomers, was subjected to the three step deprotection sequence of hydrogenation and peracetylation to give **201**. Global deprotection with hot aqueous barium hydroxide then gave *N,N*-diacetyllegionaminic acid **202** in good yield (Scheme 41).



Scheme 41: Deprotection of equatorial 2→3 galactosyl disaccharide 194 to give 202

In order to prove the regiochemistry of **195eq**, deprotection of the benzoate esters was carried out to give **203**, followed by hydrogenolysis and peracetylation. This sequence of reactions afforded a second identical sample of **202**, proving the regiochemistry of **195eq** (Scheme 42).



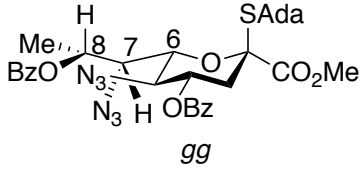
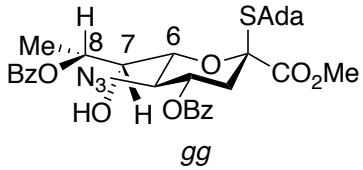
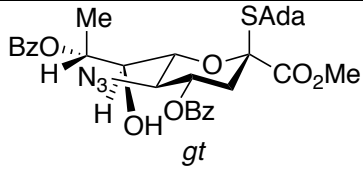
Scheme 42: Proof of regiochemistry of 195eq

2.2.13 Influence of configuration of C7 on side-chain conformation

The synthetic route adopted for the preparation of donor **181** gives us a good opportunity to evaluate the influence of C7 configuration on the side-chain conformation. Based on their $^3J_{6,7}$ values, compounds **174** and **181** retaining their D-glycero-D-galacto configuration of *N*-acetylneuraminic acid predominantly adopt the *gg*-conformation,^{143,177-182} whereas **179** differing from **174** only in C7 configuration predominantly adopts the *gt*-conformation about the exocyclic bond.^{43,144,183} Key spectral

parameters and the proposed conformations of the C7 epimers **174** and **179**, and of donor **181** are shown in the Table 5.

Table 5. Key spectral parameters and side chain conformations of **174**, **179** & **181**

Compound	Key NOE Contacts	$^3J_{6,7}$ (Hz)	C6-C7 conformation
181	H8-H6	1.4	 gg
174	H8-H6, H7-H5	≤ 1	 gg
179	H8-H5, H6-H9	2.9	 gt

2.2.14 Assignment of configuration for coupled sialosides

There are various conventional methods available for configurational assignment of aldopyranosides such as the measurement of $^3J_{H1-H2}$ and $^1J_{C1-H1}$ NMR coupling constant values. However, these methods cannot be applied to the sialic acid glycosides due to the absence of an anomeric proton. To resolve this problem in establishing anomeric configuration of sialosides, several alternative methods have been described in the literature based on

1. the chemical shift of the H-3eq,¹⁸⁴ and H-4^{185,186} resonances
2. the $\Delta\delta$ value of the resonance for H9a-9b,¹⁸⁷
3. the δ values of H-7 and H-8,^{186,187}

4. the measurement of ${}^3J_{C-1, H3ax}$ heteronuclear coupling constants^{143,172-175}

Methods 1-3 described here are based on the interpretation of chemical shift differences, which are unreliable and affected by many factors. But method 4, the measurement of ${}^3J_{C-1, H3ax}$ coupling constants, is an acceptable method as it is based on the correlation of coupling constants with torsional angle and is more reliable. This method differentiates between equatorial and axial glycosides based on the respective numerical values of 5-7 Hz and 0-2 Hz of the ${}^3J_{C-1, H3ax}$ coupling constants. The coupling constant value difference for ${}^3J_{C-1, H3ax}$ of the sialoside anomers has been rationalized by a Karplus relationship, provided the pyranose ring is in the expected 2C_5 chair conformation. In this scenario, the dihedral angles of C1-C2-C3-H3ax of the equatorial and axial anomers are 180° and 60° , respectively (Figure 9).¹⁷³

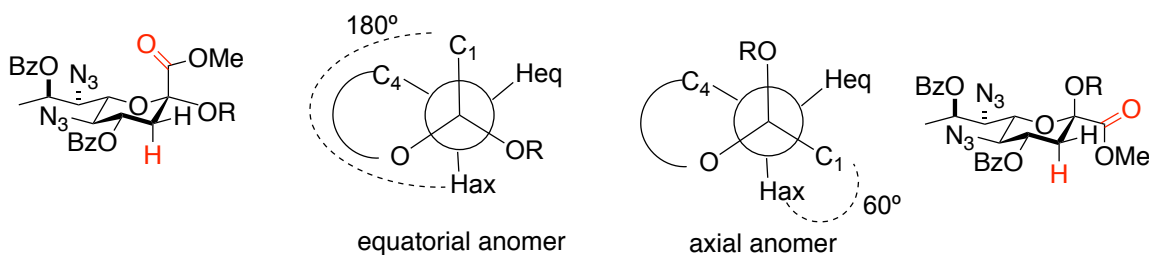


Figure 9: Dihedral angles of equatorial and axial sialosides

One example illustrating the practical implementation of the method is shown in Figure 10, whereby three different NMR experiments have been exploited to measure the ${}^3J_{C-1, H3ax}$ coupling constants.

The first experiment is the standard broadband proton decoupled ${}^{13}C$ NMR showing 5 carbonyl signals in the downfield region (166-172 ppm). The second experiment is the ${}^{13}C$ NMR spectrum with the broadband decoupler turned off showing the complete proton coupling profile for the carbonyl carbons. The most important third

experiment is the ^{13}C NMR spectrum obtained after the selective irradiation of the methyl group protons of the C-1 ester, which reduces the C-1 resonance to a doublet and provides the $^3J_{\text{C-1,H3ax}}$ coupling constant value (Figure 10).

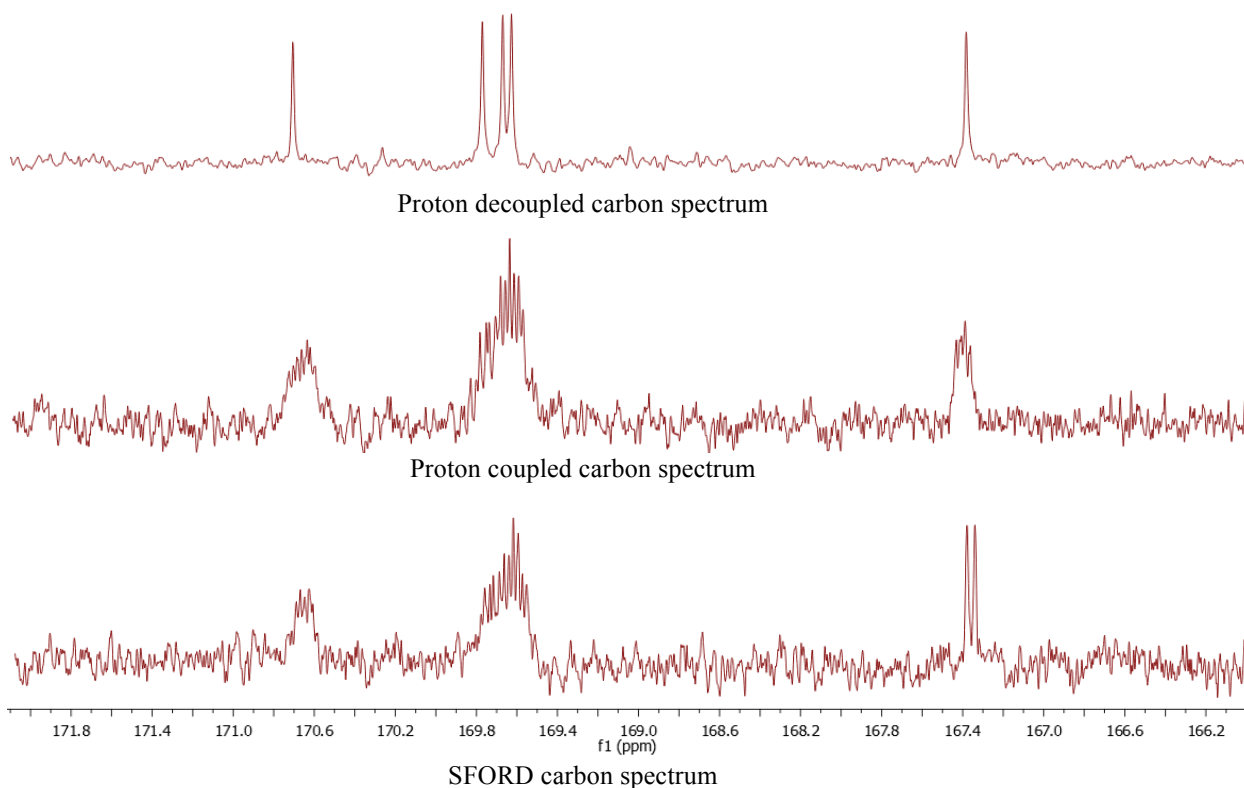
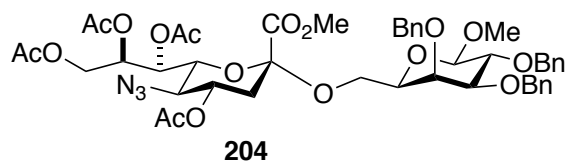


Figure 10: Example of $^3J_{\text{C-H}}$ analysis using the SFORD experiment

2.3 Conclusion

In conclusion, a simple diazido protected legionaminic acid thioglycoside donor was synthesized in 15 steps and 17% overall yield from the readily available *N*-acetylneuraminic acid. The thioglycoside donor imparted moderate to excellent glycosylation selectivity, when coupled with a range of primary, secondary and tertiary alcohols. Of particular note is the stereoselective coupling to the neuraminic acid based

acceptor **188**, whose glycosidic linkage with the donor closely resembles that found in the (2→4)-polylegionaminic acid from the lipopolysaccharide of the *L. pneumophila* virulence factor. Two of these glycosylated products were then subjected to a straightforward deprotection sequence, to finally afford the fully deprotected *N,N*-diacetyllegionaminic acid glycosides. This successful synthesis of a legionaminic acid donor and its stereoselective glycosylation reactions opened the way for the synthesis of the more complex pseudaminic acid donor, described in the next chapters.

CHAPTER 3. STEREOSELECTIVE SYNTHESIS OF EQUATORIAL 5-EPI-SIALOSIDES RELATED TO THE PSEUDAMINIC ACID GLYCOSIDES

3.1 Background

As a logical first step in a systematic investigation into the synthesis of the Pse glycosides, the synthesis of an azido based 5-*epi*-NeuAc thioglycoside donor was planned, so as to use it as a model for the glycosylation selectivity and reactivity. The use of the existing methods for the synthesis of NeuAc glycosides, especially for the equatorial glycosides, cannot be applied uncritically to the synthesis of the Leg and Pse glycosides, owing to the structural and configurational differences between Pse and NeuAc. Notably, the differences in configuration at the 5-,7-, and 8-positions and the replacement of the C-O bonds at the 7- and 9-positions by C-N and C-H bonds, respectively, in Pse potentially could all result in changes in glycosylation reactivity and selectivity. In fact, the simple inversion of configuration at the 7-position of NeuAc leads to differences in glycosylation reactivity and selectivity of O4,N5-oxazolidinone-type sialic acid donors,¹⁴³ because of the imposed change in side chain conformation from *gg*- to *gt*-¹⁴⁴ with the natural **66** being more reactive and more equatorially selective than its unnatural C-7 epimer unnatural **205** (Figure 11).

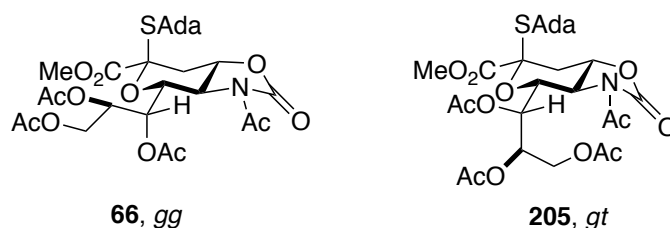


Figure 11: O4, N5-oxazolidinone sialic acid 66 and its unnatural C-7 epimer 205

This has also been shown to be the case in the previous chapter on the synthesis of legionaminic acid donor **181**, wherein a simple inversion of configuration at C7 in **179**

led to a conformational change of the side chain from *gg-* to *gt-*.¹⁶² Additionally, there is substantial literature precedent on the influence of the N5 protecting group on the glycosylation selectivity of sialic acid donors.^{111,118,130-133,188} Notably, the replacement of an equatorial substituent by the axial substituent typically affects the glycosylation reactivity and selectivity.^{149,150,189-196} Therefore, before venturing into the complex synthesis and glycosylation of a Pse donor, the synthesis of the aforementioned intermediate donor, namely an azido based *5-epi*-NeuAc thioglycoside was envisioned (Figure 12), to check the influence of the configuration at C5 on the reactivity and selectivity. Accordingly, synthesis of a *5-epi*-NeuAc thioglycoside donor and its stereoselective glycosylation reaction is the focus of the work described in this chapter.

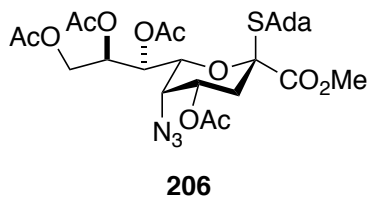
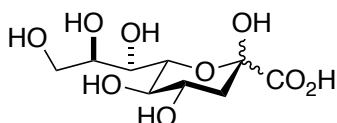


Figure 12: Planned *5-epi* NeuAc thioglycoside donor 206

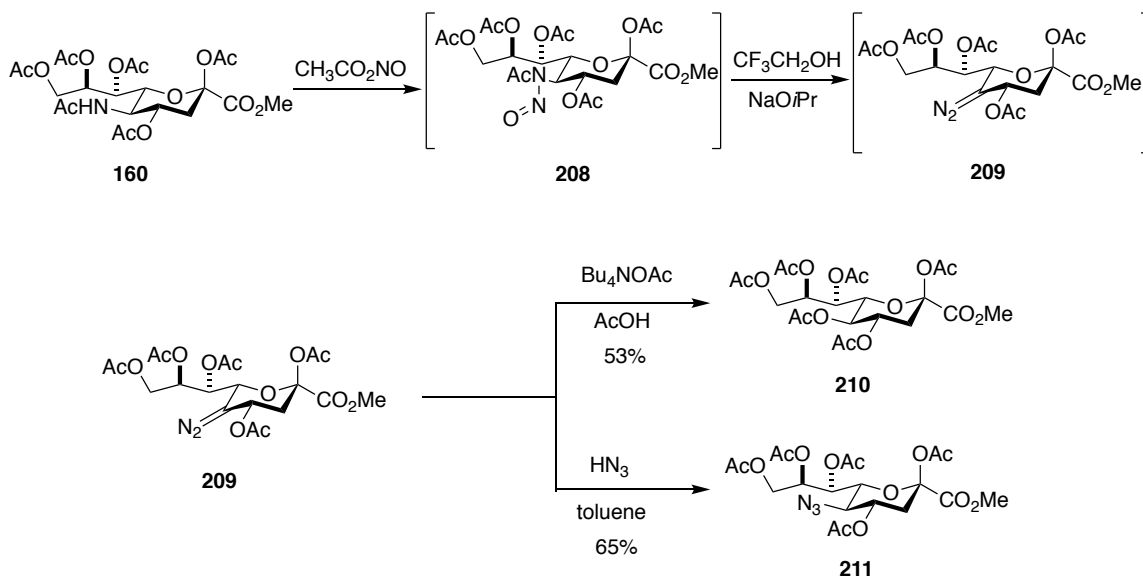
3.2 Results and Discussion

3.2.1 Zbiral oxidative deamination of a NeuAc thioglycoside with levulinic acid as nucleophile

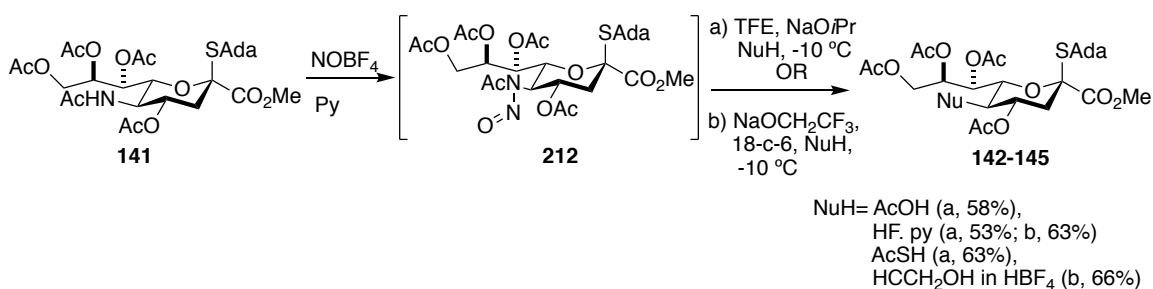
The only successful route for the inversion of configuration at C5 of *N*-acetylneuraminic acid employs Zbiral's oxidative deamination, to access a derivative of 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN, Figure 13) with retention of configuration.^{39,140}

**207****Figure 13: 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN)**

The original Zbiral deamination sequence synthesized the nitrosyl sialic acid **208** with nitrosyl acetate generated in situ from gaseous dinitrogen tetroxide and sodium acetate. The gaseous dinitrogen tetroxide needed for the reaction was generated by decomposition of lead nitrate. The nitrosyl sialic acid **208** was treated with trifluoroethanol and sodium isopropanol to generate the 5-diazo sialic acid **209** in situ, which was immediately quenched with a mixture of tetrabutylammonium acetate and acetic acid to afford the hexaacetylated sialic acid **210**. Similarly, treating the in-situ generated 5-diazo sialic acid **209** with hydrazoic acid as nucleophile furnished the 5-azido sialic acid **211** (Scheme 43).¹⁹⁷

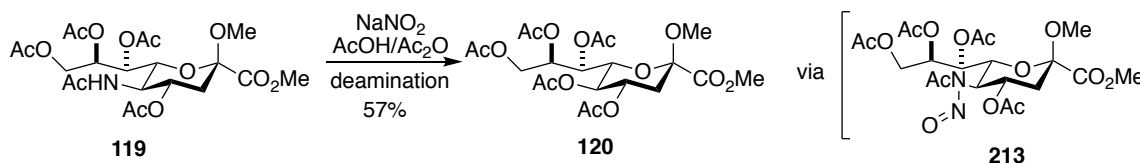
**Scheme 43: Original Zbiral deamination reaction with acetic acid and hydrazoic acid as nucleophiles**

The use of lead nitrate was not considered green, so a more environmentally friendly alternative route to access the intermediate nitrosyl sialic acid was imperative. To this end, Crich *et al.* carried out the nitrosation reaction with nitrosonium tetrafluoroborate and pyridine to give the nitrosyl sialic acid intermediate **212** in quantitative yield. This compound was then deaminated with the trifluoroethanol/sodium isopropoxide couple and acetic acid as nucleophile to access the KDN derivatives **142-145**.¹⁴⁵ The reaction was compatible with various other nucleophiles as well, such as hydrogen fluoride and thioacetic acid (Scheme 44).



Scheme 44: Crich's alternate route to generate a *N*-nitrosoamide intermediate in a modified Zbiral reaction

Starting from the 5-acetamido sialoside **119**, Kiefel *et al.* also performed a one-pot reaction with sodium nitrite and a mixture of acetic acid and acetic anhydride to synthesize the KDN derivative **120** via the nitrosyl sialic acid intermediate **213** in situ (Scheme 45).^{39,140}

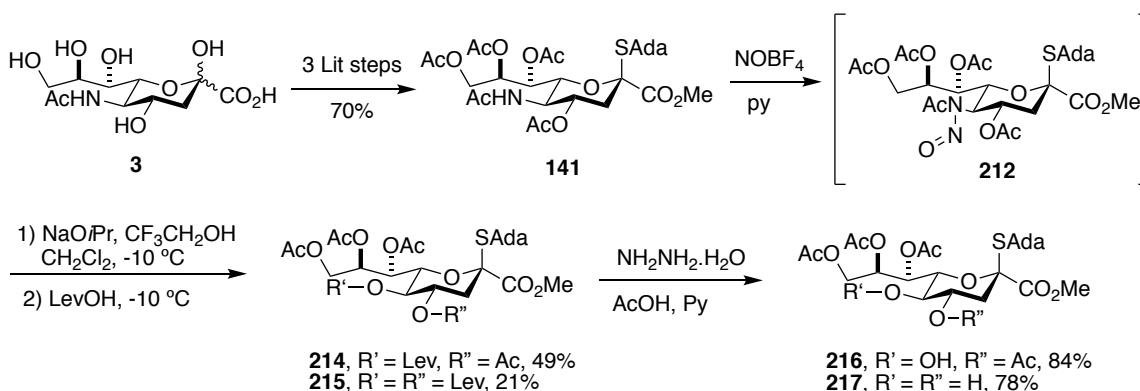


Scheme 45: Kiefel's one-pot protocol to generate *N*-nitrosamide in situ

However, a drawback to their methods was the use of acetic acid as nucleophile in oxidative deamination reaction, which resulted in the formation of the penta-acetate **120**,

lacking the differential functionalization at the 5-position. A first goal was therefore to replace acetic acid by an alternative nucleophile, so as to afford a KDN derivative with differential protection of O5.

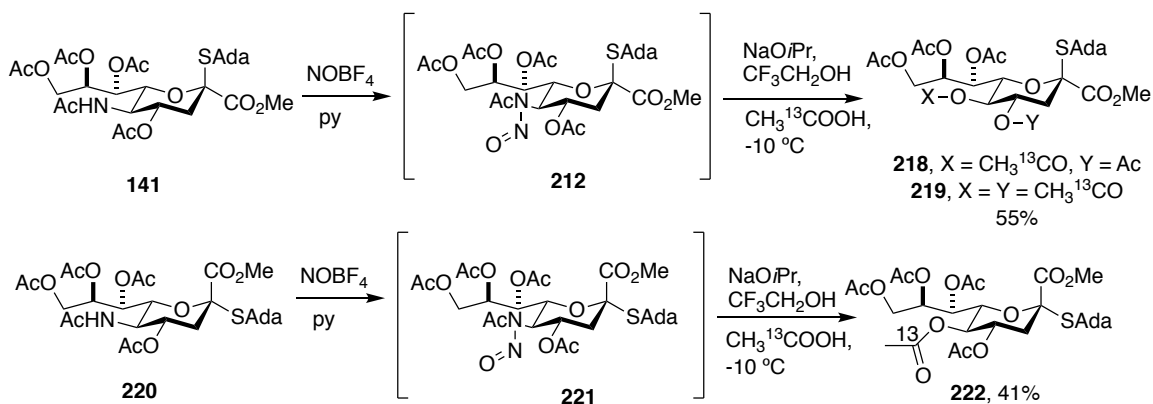
The common intermediate **141** for the synthesis of all of the sialic acid donors in this thesis was synthesized from *N*-acetylneuraminic acid in 3 steps and 70% overall yield.¹³¹ With compound **141** in hand, the synthesis of a selectively protected 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) derivative suitable for the introduction of nitrogen functionality (azido in our case) at the 5-position was explored. Accordingly, **141** was treated with nitrosonium tetrafluoroborate in the presence of pyridine in dichloromethane at -10 °C to obtain the *N*-nitroso derivative **212**. This compound was subsequently treated with levulinic acid to obtain the 5-*O*-levulinyl KDN derivative **214** in 49% yield, alongside the 4,5-di-*O*-levulinyl product **215** in 21% yield (Scheme 46). Reaction of levulinate **214** and dilevulinate **215** with hydrazine monohydrate in acetic acid and pyridine afforded the KDN-5-ol **216** and 4,5-diol **217**, thus confirming the regioselectivity of **215** in the ester exchange reaction.¹⁹⁸



Scheme 46: Modified Zbiral deamination with levulinic acid as nucleophile

The exchange of the 4-*O*-acetate with the nucleophilic carboxylate as a minor process follows same pattern the Crich laboratory has previously observed when using

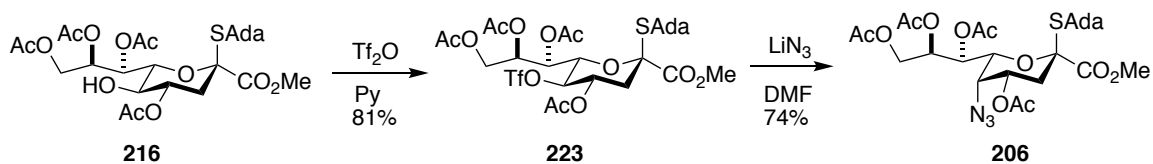
isotopically labeled acetic acid as nucleophile, whereby the exchange was observed only with the axial thioglycoside **141** and not with the equatorial thioglycoside **220** (Scheme 47).¹⁵⁹



Scheme 47: Zbiral deamination with isotopically labeled acetic acid as nucleophile

3.2.2 Synthesis of a 5-*epi*-azido donor

Compound **216** was triflated to give **223** in 81% yield, and then treated with excess lithium azide in DMF at 0 °C to afford the D-glycero-D-gulo configured 5-*epi*-NeuAc thioglycoside donor **206** in 74% yield (Scheme 48). Lithium azide was used in place of more common sodium azide, owing to the higher solubility of lithium azide in DMF used as solvent.

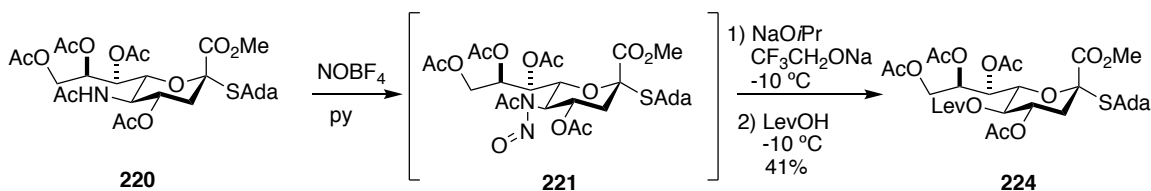


Scheme 48: Synthesis of the azido based 5-*epi*-NeuAc donor 206

3.2.3 Oxidative deamination of epimeric thioglycoside using levulinic acid

The same deamination chemistry using levulinic acid as nucleophile was used in the deamination of the diastereomeric thioglycoside **220** via the intermediate *N*-nitroso amide **221**, obtained by treatment of NOBF₄ in presence of pyridine. The intermediate

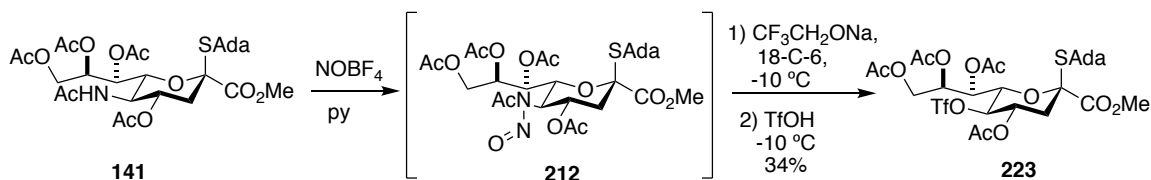
221 gave 5-*O*-levulinate **224** as a single product in 41% yield. This result confirms the previous finding¹⁵⁹ that the ester exchange between C4 and C5 occurs only in substrates having an axial thioglycoside (Scheme 49).



Scheme 49: Synthesis of the 5-*O*-levulinoyl epimeric thioglycoside 224

3.2.4 Oxidative deamination of a NeuAc thioglycoside using triflic acid as nucleophile

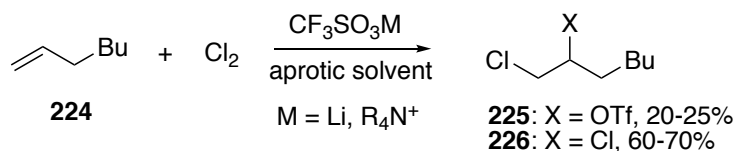
The triflated product **223** was also directly accessed from **141** using an oxidative deamination protocol with triflic acid as nucleophile in 34% yield (Scheme 50).



Scheme 50: Alternative route to access 223 via deamination reaction with triflic acid

The triflate anion is widely used as a good leaving group,^{199,200} but its use as nucleophile in substitution reactions is rarely considered, even though there are ample examples of such the literature as set out in a recent review.²⁰¹

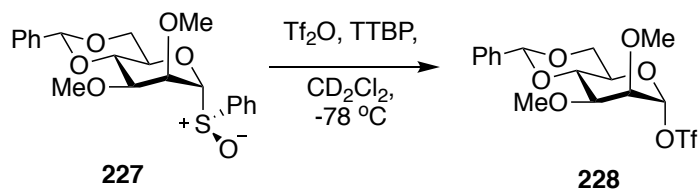
Zefirov and Kozmin have previously shown triflate to be a competitive nucleophile that can trap a variety of carbocation-like electrophiles (Scheme 51).^{202,203}



Scheme 51: Electrophilic addition on alkenes to form triflate

The high nucleophilicity of triflate toward cation-like species is also apparent from an intrinsic reactivity index developed on the basis of a computational study,²⁰⁴ whereby the ranking of reactivity $\text{Cl}^- > \text{Br}^- \gg \text{AcO}^- > \text{MeSO}_3^- > \text{TfO}^- > \text{MeOH}$, shows the triflate ion to have higher nucleophilicity than methanol.

The most common use of triflate as nucleophile in preparative chemistry is in the generation of glycosyl triflates, which are used as glycosyl donors. Applying appropriate conditions, glycosyl donors are converted to the corresponding glycosyl triflates in situ. NMR methods have been utilized in the Crich laboratory to reveal the formation of glycosyl triflates from glycosyl sulfoxides with triflic anhydride at low temperature (Scheme 52).²⁰⁵



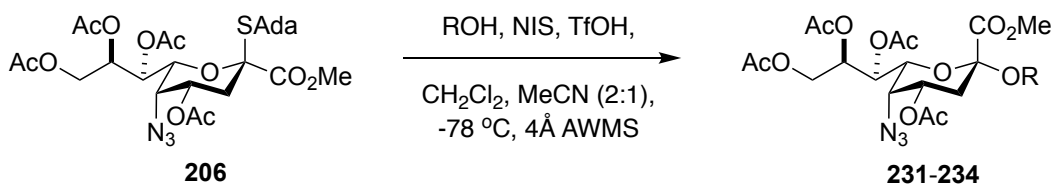
Scheme 52: Generation of mannosyl triflate from the sulfoxide

All of these reactions²⁰¹ establish the triflate anion as an excellent nucleophile toward cation-like electrophiles, and corroborate its use in the direct synthesis of **223**. The ability to isolate **223** is attributed to the two electron-withdrawing C-O bonds vicinal to the triflate which retard any subsequent displacement reactions.

3.2.5 Glycosylation study with the 5-*epi*-azido donor **206**

Similar to the case of the Leg donor **181**, the glycosylation reactions of assorted alcohols were performed with the synthesized 5-*epi*-NeuAc donor **206** by activating with *N*-iodosuccinimide and triflic acid in a 2:1 mixture of dichloromethane and acetonitrile at -78 °C in the presence of 4Å acid-washed molecular sieves (Scheme 53). The acceptors


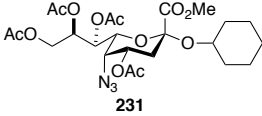
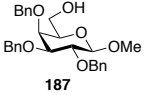
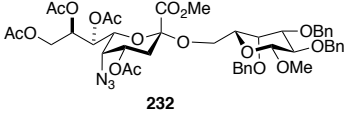
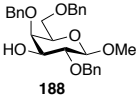
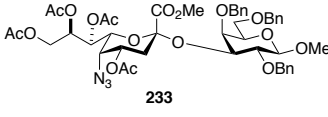
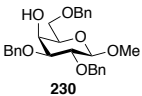
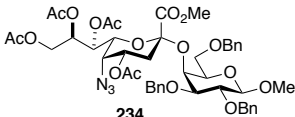
used were benzyl alcohol, methyl 2,3,4-tri-*O*-benzyl- β -D-galactopyranoside **187**, methyl 2,4,6-tri-*O*-benzyl- β -D-galactopyranoside **189** and methyl 2,3,6-tri-*O*-benzyl- β -D-galactopyranoside **230**. The linkages obtained from the galactopyranosyl 6- and 3-alcohols **187** and **189** are the most common linkages found in the sialic acid glycosides, hence the selection of these alcohols as acceptors for the glycosylation study. The choice of highly hindered galactopyranosyl 4-alcohol **230** arose from the presence of the Pse-eq.(2 \rightarrow 4)-6-deoxy-*N*-acetylgalactosamide linkage in the repeating unit of the *Pseudomonas aeruginosa* O10 lipopolysaccharides.⁷⁷



Scheme 53: Glycosylation with the 5-*epi*-NeuAc donor **206**

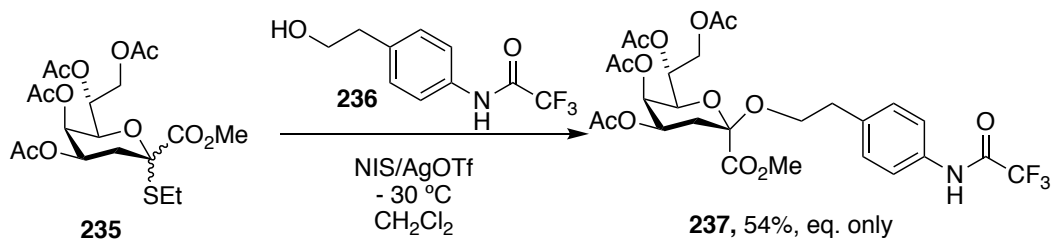
Glycosylation reactions with all the acceptors including the hindered secondary alcohol **230** gave the anticipated products in the form of single equatorial anomers, in good to excellent yields as reported in Table 6.

Table 6. Glycosylation Reactions with D-Glycero-D-gulo Donor **206**.

Entry	Acceptor	Product	Yield	$^3J_{C1,H3}$ (Hz)
1	 229	 231	79%	6.9
2	 187	 232	72%	6.6
3	 188	 233	57%	7.1
4	 230	 234	44%	7.2

3.2.6 Use of 5-azido as protecting group in sialyl donors

The excellent selectivity observed with the 5-*epi* NeuAc donor **206** compares well with similar equatorial selectivity reported by Oscarson *et al.* with the KDO-based thioglycoside donor **235** having axial 5-acetoxy group,²⁰⁶ of which example is shown below in Scheme 54.



Scheme 54: Oscarson's stereoselective glycosylation with the KDO based 5-*epi* acetoxy donor **235**

With the 5-*epi* azido protected thioglycoside donor itself, there is no literature precedent for the glycosylation reaction, indeed there was no previous synthesis of a 5-*epi* donor. However, there are ample examples in the literature of the synthesis of the equatorial 5-azido protected NeuAc donors, although the glycosylation stereoselectivity has been mostly observed with the primary alcohols as acceptors only. The various 5-azido protected NeuAc donor and an isothiocyanato protected NeuAc donor described in the literature are shown below (Figure 14).

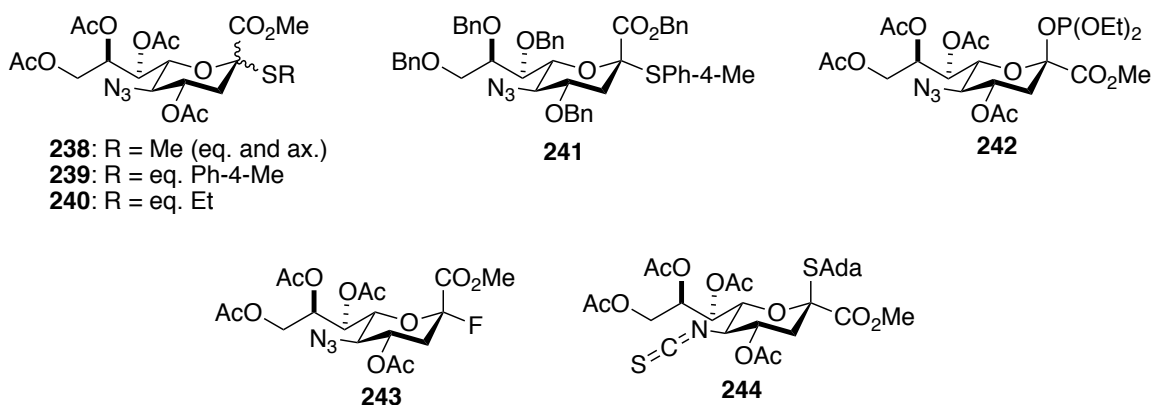
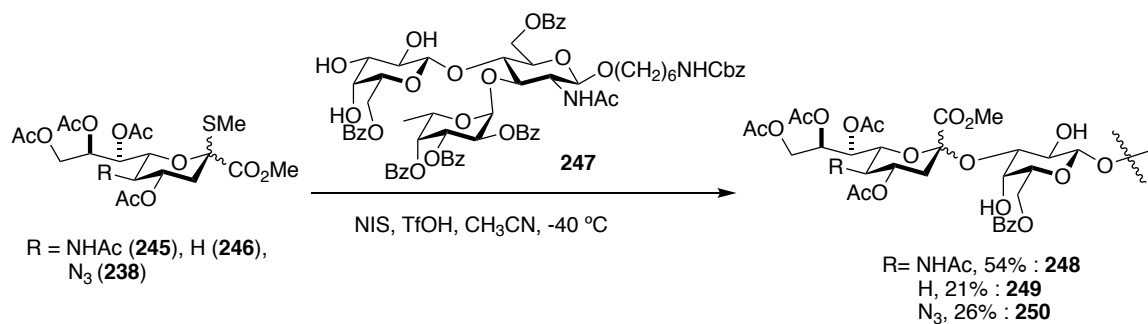


Figure 14: Literature 5-azido and 5-isothiocyanato NeuAc donors

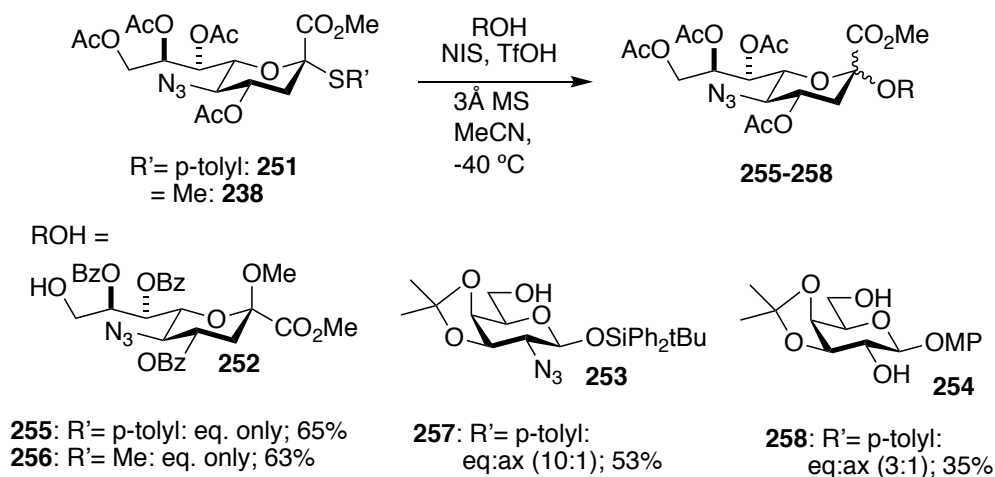
Schmidt *et al.* synthesized the ethyl thioglycoside **240**, but didn't follow up with any glycosylation reactions.¹¹⁴ The following five laboratories did synthesize 5-azido protected NeuAc donors and study their glycosylation reactions as summarized below.

- i) Unverzagt *et al.* reported the synthesis of the the 5-azido methyl thiosialoside **238** as an equatorial and axial mixture, alongside the 5-acetamido methyl thiosialoside **245**.²⁰⁷ The acceptor used was the complex trisaccharyl galactosyl 3-OH acceptor **247**, with the activation being achieved at -40 °C in acetonitrile. Unfortunately, this reaction gave only 26% of the tetrasaccharide **250**, which was worse than the coupling between the 5-acetamido donor **245** and the acceptor **247** (54% yield), and there was no discussion of their stereoselectivity (Scheme 55).



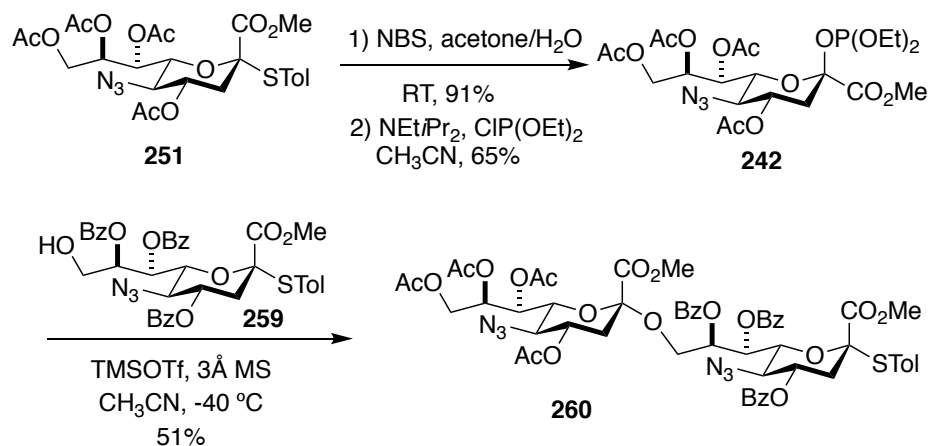
Scheme 55: Unverzagt's glycosylation reactions with various NeuAc donors

- ii) The equatorial anomers of **238** and the p-tolyl thioglycoside **251** were reported by the Wong group.¹¹⁵ Donors **238** and **251** were coupled with various primary and secondary alcohols in acetonitrile at -40 °C. However, good equatorial selectivity was observed only with the carbohydrate based primary acceptors **252**, **253** and **254** (Scheme 56).



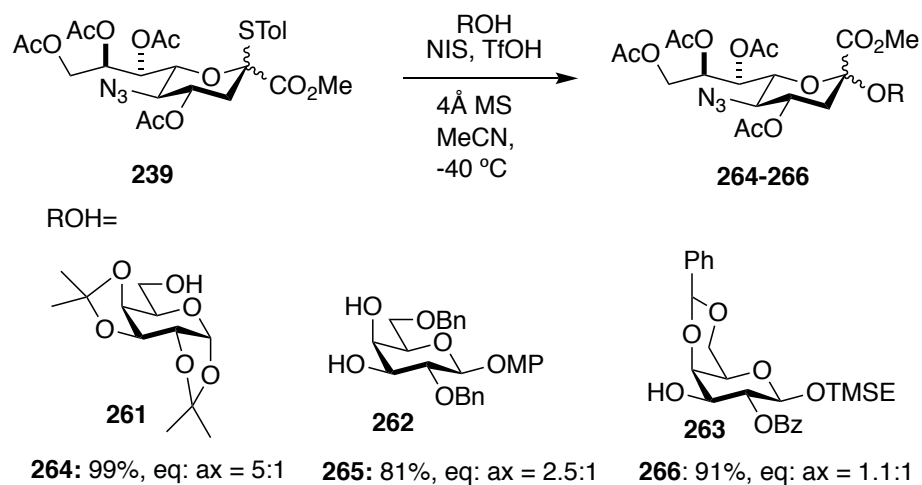
Scheme 56: Wong's glycosylations with 5-azido NeuAc donors **238** and **251**

The Wong group also reported the synthesis of the sialyl phosphite donor **242**, starting from **251**. This donor **242** was also selective toward the primary acceptor **259** in acetonitrile at -40 °C. However, there are no reports of its coupling with secondary alcohols (Scheme 57).



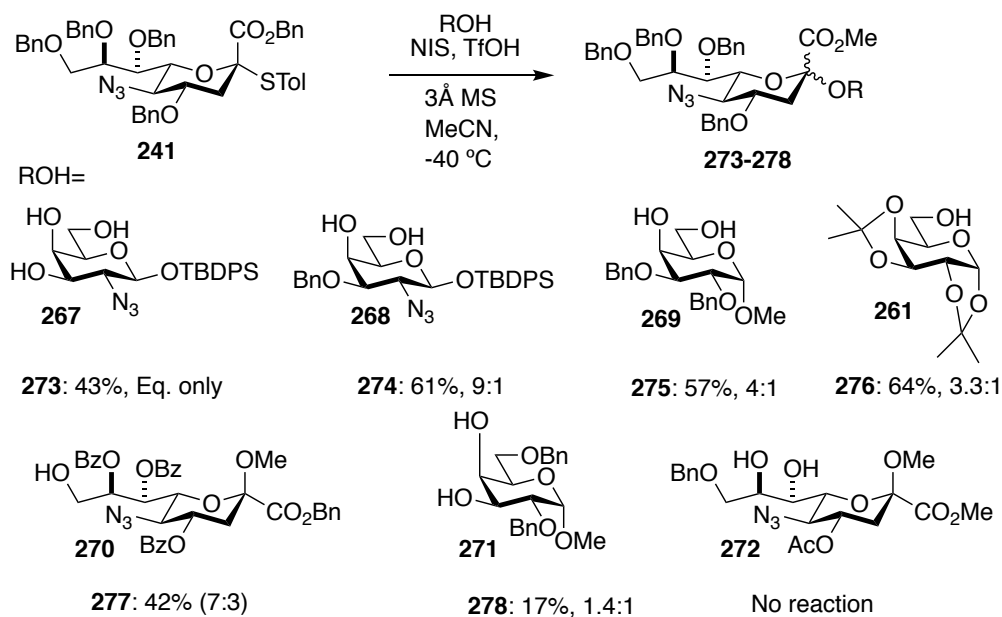
Scheme 57: Wong's glycosylation with the sialyl phosphite donor **242**

- iii) The synthesis and glycosylation of **239** from the Li group also suffered the same fate of being somewhat selective to the primary acceptors, but not when coupled with the secondary acceptors (Scheme 58).¹¹⁶



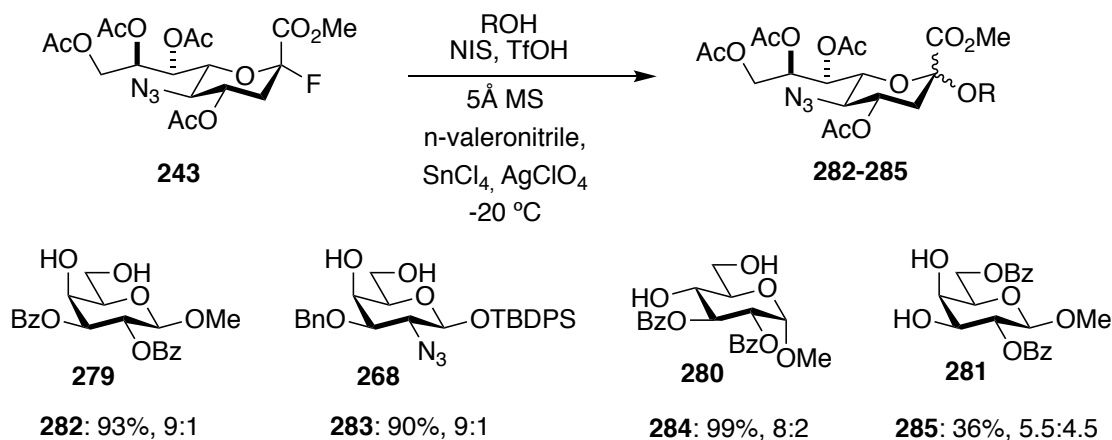
Scheme 58: Li's glycosylations with the 5-azido NeuAc donor **239**

- iv) Lin *et al.* reported the synthesis of the more highly armed thioglycoside **241**, which gave excellent to moderate selectivities with the primary acceptors **261**, **267-270**. The yield and selectivity, however, were significantly lower with the secondary acceptor **271**, and no reaction occurred with a sialic acid-based secondary acceptor **272** (Scheme 59).¹¹⁷



Scheme 59: Lin's glycosylation with the armed thioglycoside donor **241**

- v) Mukaiyama *et al.* reported the synthesis of the sialyl fluoride donor **243**. On reaction with the primary acceptors **268**, **279** and **280** at -20 °C in n-valeronitrile, it gave excellent yields and selectivities. With the secondary acceptor **281** only a low yield and minimal selectivity were observed (Scheme 60).²⁰⁸



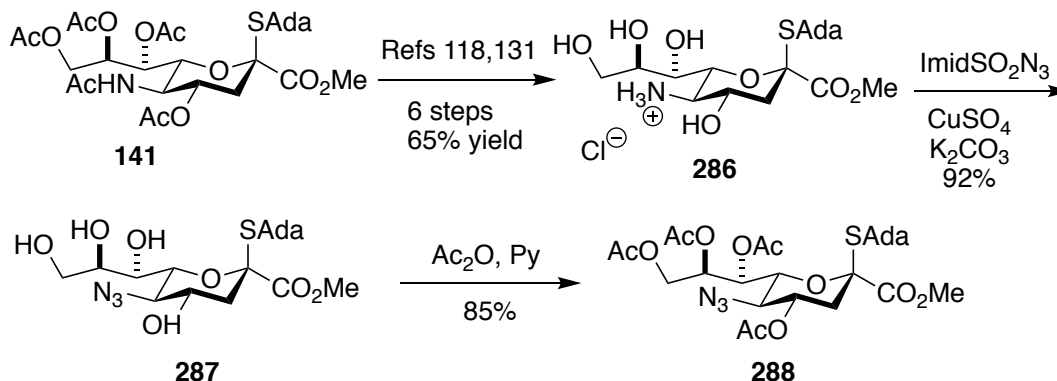
Scheme 60: Mukaiyama's glycosylation with the sialyl fluoride donor **243**

Since all of these literature azido protected NeuAc donors were found to be selective with primary acceptors only, the excellent results obtained with the donor **206** stimulated

reexamination of the use of the N5 azide in the formation of NeuAc glycosides. Accordingly, synthesis of the D-glycero-D-galacto configured NeuAc donor **288** was attempted.

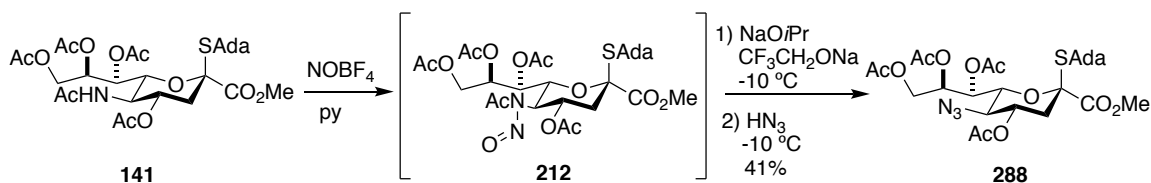
3.2.7 Synthesis of a 5-azido NeuAc donor

The common intermediate **141** for the synthesis of all the sialic acid donors was converted to the corresponding 5-amino thioglycoside derivative **286** in 6 known steps and 65% overall yield.^{118,131} Reaction of the 5-amino thioglycoside **286** with Stick's reagent¹⁶⁵ in the presence of copper sulfate and potassium carbonate gave the 5-azido protected NeuAc thioglycoside **287** in 92% yield, which on acetylation gave the desired tetraacetylated 5-azido protected NeuAc donor **288** in 85% yield (Scheme 61).



Scheme 61: Synthesis of the 5-azido thioadamantanyl NeuAc donor 288

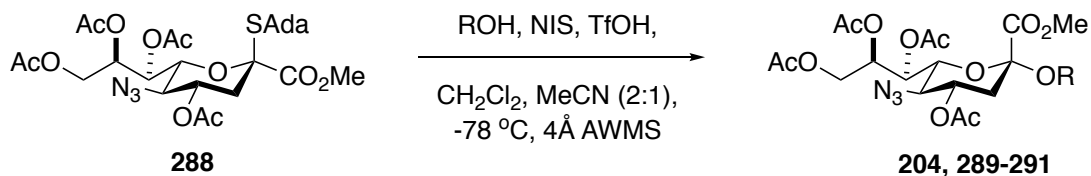
Alternatively, **288** was also accessed directly from **141**, making use of the oxidative deamination protocol with hydrazoic acid as nucleophile in 51% yield (Scheme 62).



Scheme 62: Alternate route to access donor 288 via deamination reaction

3.2.8 Glycosylation study with the 5-azido NeuAc donor **288**

Glycosylation reactions of various primary and secondary alcohols **187**, **188**, **229** and **230** with the 5-azido NeuAc donor **288** were performed using the same temperature, activation method and mixture of solvents, as previously employed with the 5-*epi*-NeuAc donor **206** (Scheme 63).

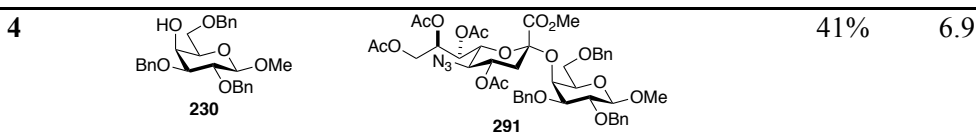


Scheme 63: Glycosylation with the 5-azido NeuAc donor **288**

Gratifyingly, the 5-azido NeuAc donor **288** was also exquisitely selective in its reactions with all the acceptors, including the hindered secondary acceptor **230**, in line with the glycosylation outcome of 5-*epi*-NeuAc donor **206** (Table 7).

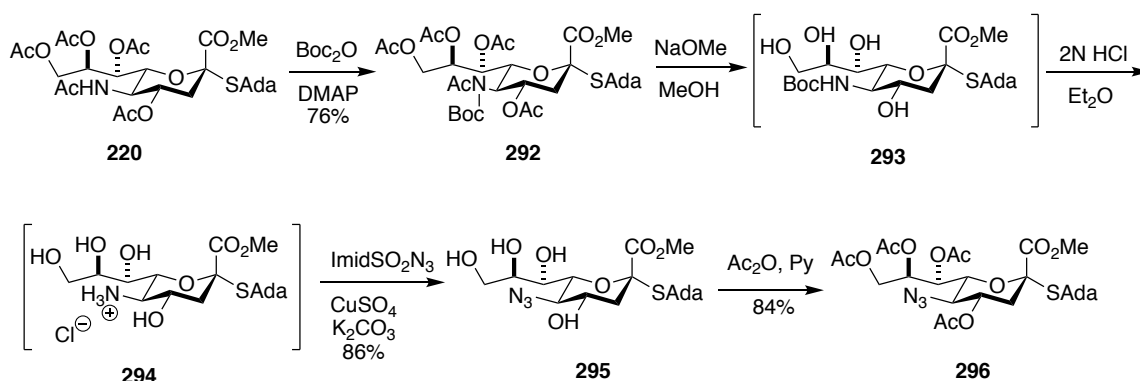
Table 7. Glycosylation Reactions with D-Glycero-D-galacto Donor **288**

Entry	Acceptor	Product	Yield	³ J _{C1,H3} (Hz)
1			81%	6.2
2			74%	6.4
3			66%	6.8



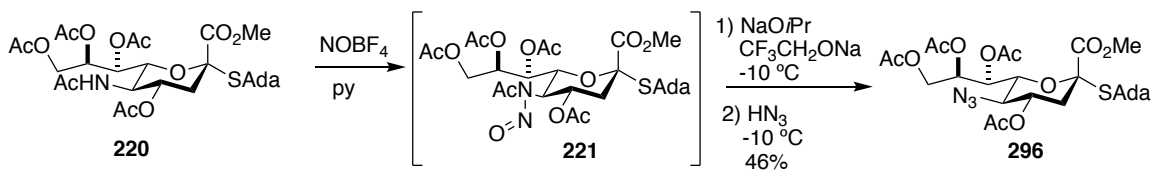
3.2.9 Synthesis of the 5-azido equatorial thioglycoside donor

There was a possibility that the axial anomeric configuration of donors **206** and **288** was playing a role in the glycosylation selectivity. To check it, the anomer of the donor **288**, the 5-azido equatorial thioglycoside donor **296** was synthesized, adapting the same protocol applied for the preparation of **288**.¹³¹ Thus the intermediate **292** was subjected to the three-step sequence of Zemplen deacetylation, Boc deprotection and azide formation to give **295** in 86% overall yield via the intermediates **293** and **294**. Thereafter, acetylation of **295** readily afforded the desired 5-azido equatorial thioglycoside donor **296** in 84% yield (Scheme 64).



Scheme 64: Synthesis of the equatorial thioglycoside donor 296

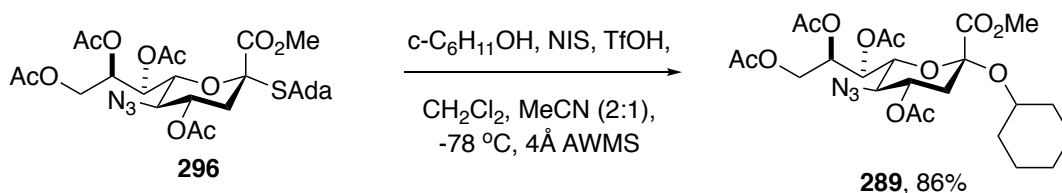
Again, using the same protocol applied for its anomer, **296** was alternatively accessed from **220** via the *N*-nitrosoamide **221** by the Zbiral deamination protocol using hydrazoic acid as nucleophile (Scheme 65).¹⁹⁷



Scheme 65: Alternate route to access the donor 296 via deamination reaction

3.2.10 Stereoselective glycosylation with the equatorial thioglycoside donor 296

The equatorial thioglycoside donor **296** was then subjected to glycosylation reaction with cyclohexanol as acceptor in the usual conditions of 2:1 dichloromethane/acetonitrile at $-78\text{ }^{\circ}\text{C}$ with activation by *N*-iodosuccinimide and triflic acid. The glycosylation reaction furnished the equatorial glycoside **289** as a single diastereomer in 86% yield. Since both anomer of the donors, **206** and **288**, gave equatorial glycoside **289** with comparable yields and selectivities, it was concluded that the equatorial selectivity observed with the donor **288** is not a function of the initial anomeric configuration (Scheme 66).



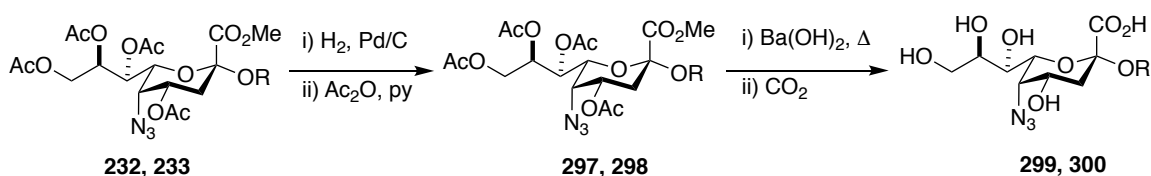
Scheme 66: Stereoselective glycosylation with the equatorial thioglycoside donor 296

The clear difference between the literature results and the results in Table 6 is the reaction temperature, leading to the conclusion that the equatorial selectivity can be achieved with such azide protected peracetyl sialyl donors if the glycosylation reactions are performed at $-78\text{ }^{\circ}\text{C}$. Therefore, it appears likely that if the literature donors **238-244** are to subject to the glycosylation reactions at $-78\text{ }^{\circ}\text{C}$, they would also be equatorially selective. Similar glycosylation selectivities with primary and secondary alcohols in

acetonitrile/dichloromethane mixtures were obtained by the Crich laboratory with the isosteric isothiocyanate protected sialyl donor **244** at $-78\text{ }^{\circ}\text{C}$,¹¹⁸ suggesting that there is virtually no difference between the isosteric azide and isothiocyanato groups if the reactions are performed at the same low temperature conditions.

3.2.11 Deprotection of 5-*epi*-azido glycosides

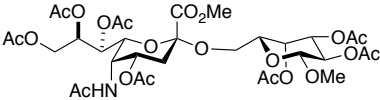
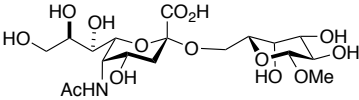
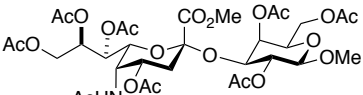
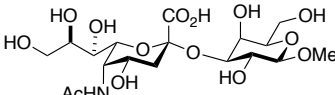
Deprotections of two of the 5-*epi*-azido NeuAc glycosides, viz. **232** and **233** were carried out following a 3-step deprotection sequence (Scheme 67).



Scheme 67: Deprotection protocol of the 5-*epi* azido glycosides **232** and **233**

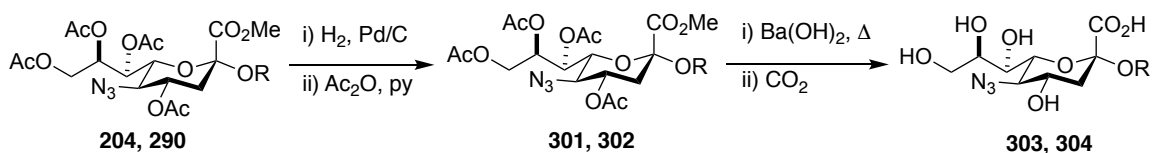
Initially, hydrogenolysis over palladium-charcoal in aqueous dioxane was performed which resulted in the cleavage of the benzyl ethers and the reduction of the azido groups to the corresponding amines. Partial O \rightarrow N migration of the acetyl groups was observed at this step,^{124,209} therefore no attempt was made to purify the products. Consequently, global acetylation of the crude products afforded the peracetate containing acetamides **297** and **298**, respectively in high yields. Both intermediates **297** and **298** were subjected to global deprotection of all the esters using hot aqueous barium hydroxide to give the final deprotected glycosylated products **299** and **300**, respectively, in high yields (Table 8).

Table 8. Deprotection of the 5-*epi* azido glycosides 232 and 233

Substrate	Hydrogenolysis Product, % yield	Sialic Acid Glycoside, % yield
22	 297, 82%	 299, 92%
23	 298, 77%	 300, 93%

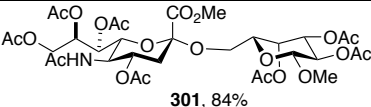
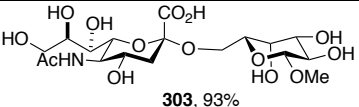
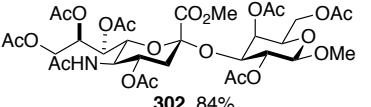
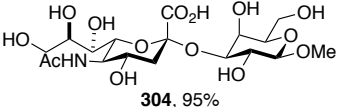
3.2.12 Deprotection of 5-azido glycosides

The same reaction sequence of hydrogenolysis over palladium-charcoal in aqueous dioxane, peracetylation and global deprotection of esters using hot aqueous barium hydroxide for the 5-*epi*-azido NeuAc glycosides was applied to the isomers at 5-position, **204** and **290** (Scheme 68).

**Scheme 68: Deprotection protocol of the 5-azido glycosides 204 and 290**

This deprotection sequence gave the final deprotected products **303** and **304** via the respective intermediates **310** and **311** (Table 9).

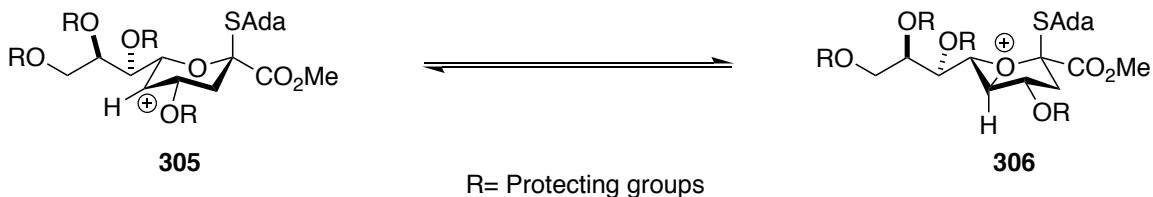
Table 9. Deprotection of the 5-azido glycosides 204 and 290

Substrate	Hydrogenolysis Product, % yield	Sialic Acid Glycoside, % yield
29	 301, 84%	 303, 93%
30	 302, 84%	 304, 95%

3.2.13 Leaving group-dependent stereoselectivity in substitution at the 5-position of NeuAc

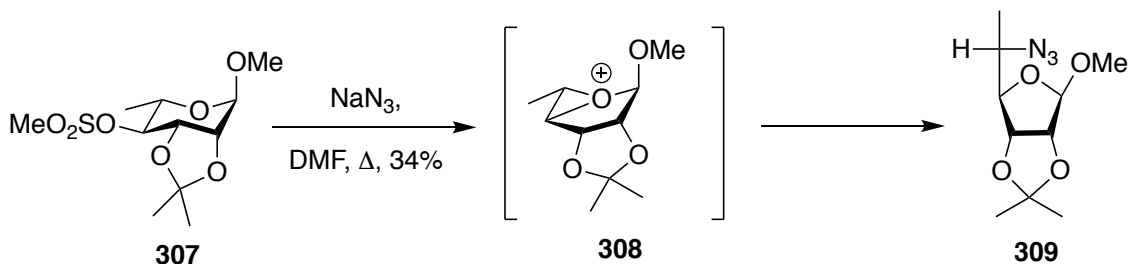
It is noteworthy that the oxidative deamination of **141** and related substances proceeds with the retention of configuration, in line with the results observed by Zbiral and other groups. Whereas the similar substrate **223** gave the stereoinverted azido product **206** when triflate was displaced by azide. The previous experimental analyses done by the Crich laboratory suggest that the stereoretentive property of the oxidative deamination reaction is because of the participation of the pyranoside ring oxygen to displace the molecular nitrogen via the oxabicyclo[3.1.0]hexanium ion **306**.¹⁵⁹ This particular ring oxygen intervention is dependent upon the nature of the leaving group. The extreme leaving group ability of molecular nitrogen is facilitating the participation of the pyranoside ring oxygen, either directly or after the formation of the intermediate carbenium ion **305**.^{196,197} This is then only followed by the intermolecular attack by external nucleophiles such as azide or acetate. Whether the reaction equilibrium favors the carbenium ion or oxobicyclo[3.1.0]hexanium ion depends upon the nature of the –OH

protecting groups. If the hydroxy protecting groups are electron withdrawing groups, such as acetates, the equilibrium favors bicyclic oxonium ion, which leads to the stereoselective deaminated products. However, if the protecting groups are less electron withdrawing, such as benzyl ethers or isopropylidene groups, the reaction equilibrium favors carbenium ion, which leads to unselective deaminated products (Scheme 69).



Scheme 69: Equilibrating carbenium and bridged oxonium ions

This participation of ring oxygen doesn't occur in the S_N2 displacement of triflate by azide in the formation of **206**, because though it is a good leaving group, is not as excellent a leaving group as molecular nitrogen is. Participation by the ring oxygen may become possible if the displacement of the sulfonate by external nucleophiles is sterically hindered, such as in the cases described by Stevens and Hanessian laboratories in a series of pyranose to furanose ring contractions,²¹⁰⁻²¹² one of the example is shown in Scheme 70. Ring contraction in the oxidative deamination of an *N*-acetylneuraminic acid glycoside has also been reported, albeit only as a minor product.²¹³



Scheme 70: Ring contraction of a rhamnosyl 4-*O*-sulfonate

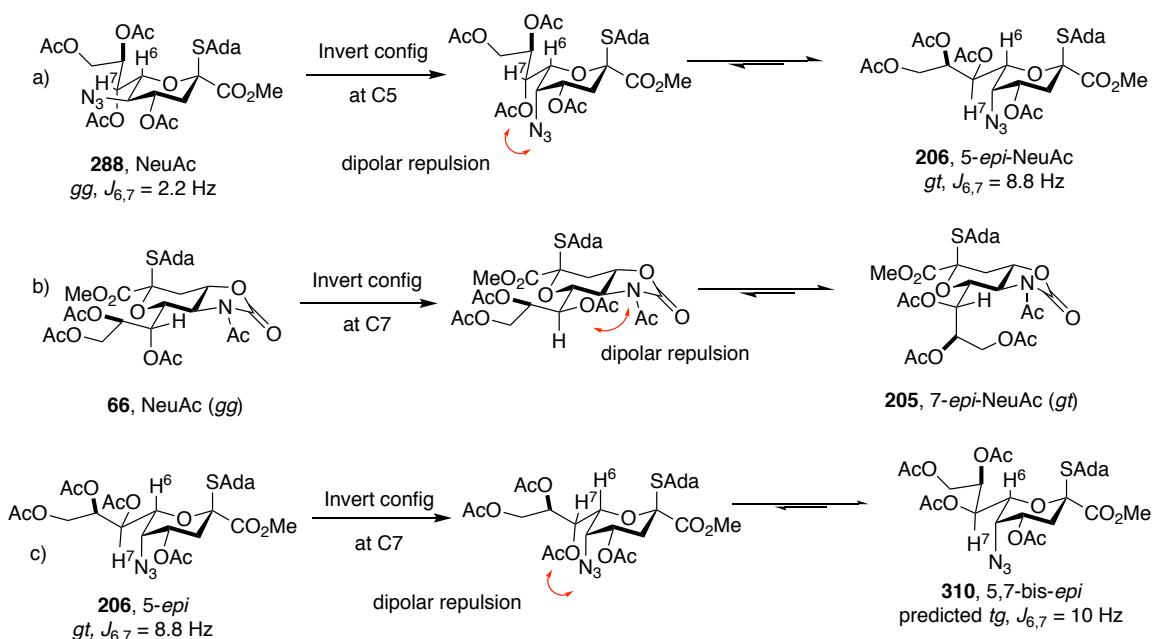
3.2.14 Influence of the C5 configuration on side-chain conformation and glycosylation selectivity

Sialic acid donors with an axial C-N bond at the 5-position were initially expected to give a greater proportion of axial glycosides than their C-5 epimers. The axial C-N bond has a gauche relationship to the ring oxygen whereas the equatorial C-N bond has antiperiplanar relationship to the ring oxygen. Therefore the axial C-N bond is better able to stabilize the developing positive charge at the ring oxygen during glycosylation reaction.^{149,193} Per this argument, donor **206** should have been less equatorially selective than the donor **288**. The comparable equatorial selectivity observed in their glycosylation reactions at -78 °C in spite of their differing C-5 configurations provoked close inspection of the H6-H7 coupling constant values in their ¹H NMR spectra, leading to the conclusion that donors **206** and **288** adopt different conformations about the exocyclic bond to the side chain. Donor **288** has a ³J_{6,7} coupling constant of 2.2 Hz consistent with the predominant *gg*-conformation of the side chain,¹⁵¹ in line with the previous analyses of the derivatives of *N*-acetylneuraminic acid.¹⁴³ On the other hand, donor **206** has ³J_{6,7} coupling constant of 8.8 Hz, indicating H6 and H7 to be antiperiplanar and the side chain to adopt the *gt*-conformation. The transformation from the predominant *gg*- to *gt*-conformation for the donor **206** must arise from a combination of dipolar and steric interactions between C5-N5 bond and the side chain in the *gg*-conformation (Scheme 71a). Seminal work from the Bols group has shown that donors with the *gt*-conformation are less reactive,²¹⁴ and therefore have less chance to form axial glycosides than their *gg*-isomers.¹⁴⁴ Hence, it seems that for the donor **206**, the negative effect of having an axial C5-N5 bond is offset by the positive effect of the conformational change from *gg* to *gt*

about the the exocyclic bond to the side chain, providing the comparable selectivities between donors **206** and **288**.

Furthermore, the previous investigation on the influence of configuration at the 7-position with the NeuAc and the 7-*epi*-NeuAc donors **66** and **205**, respectively has also shown that the 7-*epi*-NeuAc donor **205** exhibit a different side chain conformation, namely the *gt*-conformation, again in order to avoid unfavorable dipolar and steric interactions with the C5-N5 bond (Figure 71b).¹⁴³ The same case of conformational change from the predominant *gg*- to *gt*-conformer was observed between **174** and its C-7 epimer **179**, intermediates in the synthesis of the legionaminic acid donor **181**.¹⁶²

Extrapolating these results, it is predicted that the inversion of both the configurations at C-5 and C-7, as that of pseudaminic acid, would again result in unfavorable dipolar and steric repulsion in the hypothetical 5,7-bis-*epi* system **310** to the highest level, due to which the side chain conformation would change from the predominant *gt* to the most-disarming and the least active *tg*-conformer (Scheme 71c).



Scheme 71: Influence of C5 and C7 configuration on the side chain conformation

Therefore, compared to the moderately equatorial selective Leg donor **181**, the planned pseudaminic acid donor **311** would be expected to adopt the *tg*-conformation very predominantly and as a result of which is predicted to afford excellent equatorial selectivity in its glycosylation reactions (Figure 15).

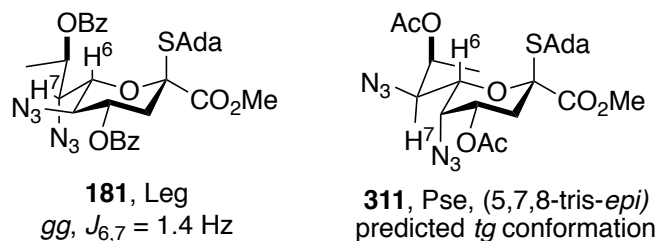


Figure 15: Equatorially selective Leg and Pse donors

3.3 Conclusion

In conclusion, the D-glycero-D-gulo configured azido based 5-*epi*-NeuAc donor **206** and its C-5 epimer D-glycero-D-galacto configured azido based NeuAc donor **292** were synthesized and subjected to glycosylation reactions at -78 °C. The exquisite equatorial selectivity obtained from both the donors **206** and **288** suggested that the azido group at the 5-position assisted in the equatorial glycosylation selectivity provided that the reactions are carried at -78 °C.

The observed glycosylation selectivity of 5-*epi*-NeuAc donor was attributed to the conformational change about the exocyclic bond to the side chain from the predominant *gg*- to *gt*-conformation, which offset the presence of the axial azide in the pyranose ring. Therefore, it was concluded that if a suitable pseudaminic acid donor with azido protecting group could be synthesized, there would be a high possibility of obtaining its equatorial glycosides on glycosylation at -78 °C. Another significant aspect was the observation of the triflate anion as a nucleophile in Zbiral deamination reaction, which

affirmed the previous analyses of its excellent nucleophilicity toward carbenium ion like electrophiles.

CHAPTER 4. SYNTHESIS AND STEREOCONTROLLED EQUATORIALLY SELECTIVE GLYCOSYLATION REACTIONS OF A PSEUDAMINIC ACID DONOR

4.1 Background

Pseudaminic acid was first identified by Knirel and co-workers in the lipopolysaccharides of *Pseudomonas aeruginosa* O7/O9 and *Shigella boydii* type 7 in 1984.⁵² As with legionaminic acid, its late discovery is attributed to its low abundance in nature and the earlier lack of powerful glycoanalysis tools. With the recent improvements in analytical tools, pseudaminic acid and its derivatives have been reported in other bacterial glycoconjugates such as the LPS O-antigens, capsular polysaccharides, pili and flagella of numerous other Gram-negative pathogens,^{28,57} such as *Vibrio cholera*,⁸⁵ *Campylobacter jejuni*,^{64,65} *Campylobacter coli*,⁶⁶ *Vibrio vulnificus*⁷³ and *Pseudoalteromonas distincta*.⁸⁴ In particular, *Pseudomonas aeruginosa* is a notorious pathogen found in the sufferers of cystic fibrosis and various gastrointestinal diseases. The naturally occurring pseudaminic acid and its derivatives have been found to contain linkages through the nitrogen functionalities at C-5 and C-7. Two such examples are the C-7 linked pseudaminic acid derivative **30** found in *Kribella spp.*,⁵⁷ and the equatorially linked lipopolysaccharide **27** of *Pseudomonas aeruginosa* O10 (Figure 16).⁷⁷ Therefore, pseudaminic acid glycosides can be considered as the candidates for use in diagnostic tools and in the development of antibacterial vaccines. For this to happen, practical routes to pseudaminic acid donors are needed, as Pse is presently not available in sufficient quantities from natural sources.

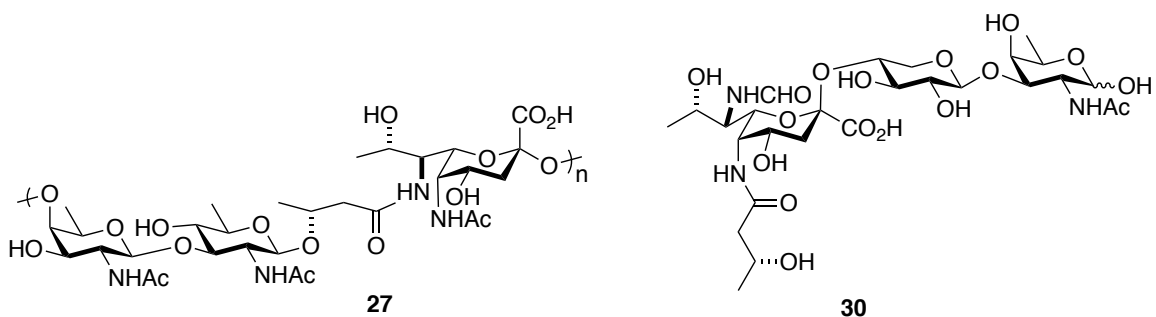


Figure 16: Naturally occurring Pse glycosides 27 and 30 containing differential nitrogen functionalities at C-5 and C-7

Tsvetkov *et al.* reported the synthesis of Pse by chain extension of a hexose sugar, but did not study its glycosylation reactions.¹³⁸ Ito *et al.* employed a similar approach to synthesize the Pse donor **116** starting from the *N*-acetylglucosamine **82**.¹³⁹ However, a glycosylation reaction of a primary acceptor with the donor **116** in acetonitrile at 0 °C resulted in axial selectivity. Kiefel *et al.* reported the synthesis of Pse from *N*-acetylneuraminic acid, but did not convert it to a donor suitable for glycosylation reactions.^{39,140} Of late, Li *et al.* reported the *de novo* synthesis of the Pse donor **130** starting from L-threonine, and found it to show axial selectivity in dichloromethane at -78 °C.¹⁴¹ Two of these literature Pse donors, **116** and **130**, and the proposed donor **311** targeted in this investigation are shown in Figure 17.

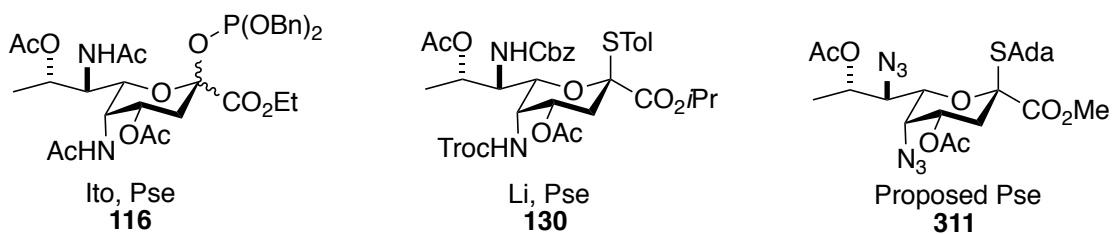
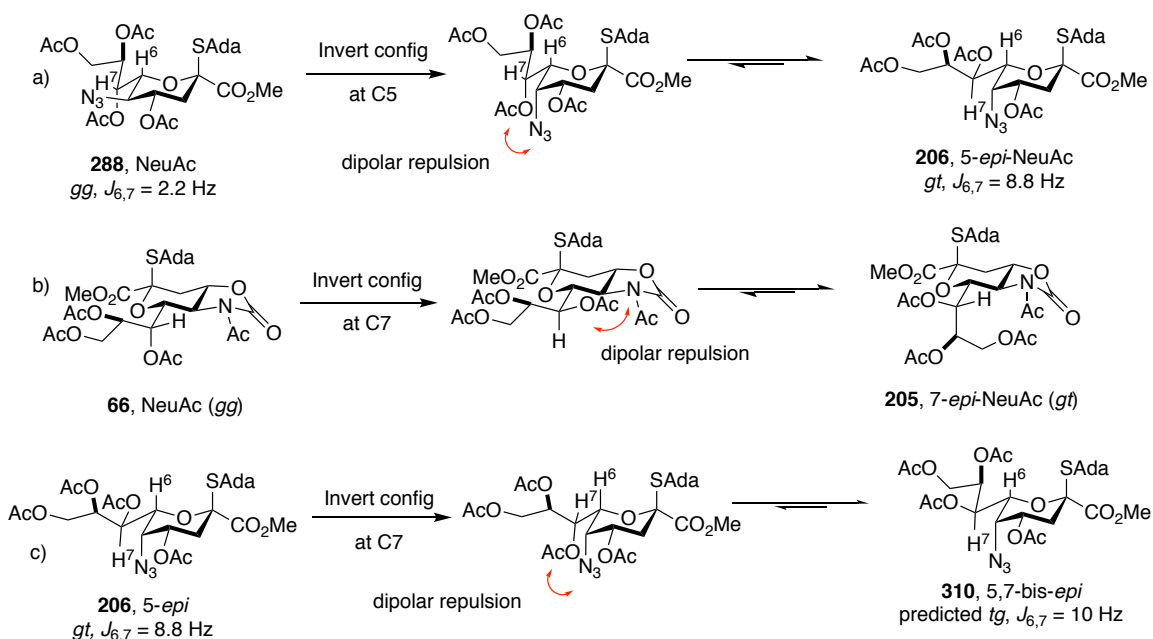


Figure 17: Literature Pse donors and the planned Pse donor

In addition to the efficient synthetic routes for pseudaminic acid donors, improved understanding of the stereoselectivity of glycosylation is needed. Minor changes in structure and conditions can lead to changes in glycosylation selectivity due to major

shifts in mechanism at the interface between S_N1 and S_N2 reactivity.²¹⁵⁻²¹⁷ Identifying the ideal donor structure and optimum reaction conditions would lead to efficient stereoselective synthesis of the sialic acid glycosides. As such, one of the interests of the Crich laboratory has been the stereoselective synthesis of the equatorial sialic acid glycosides,^{118,130,131,145,218} and understanding the role the side chain conformation plays in anomeric reactivity and selectivity.^{43,143,144,162,219,220} As reported in chapter 3, it was found that the 5-*epi*-NeuAc donor **206** has $^3J_{6,7}$ coupling constant of 8.8 Hz, indicating H6 and H7 to be antiperiplanar and the side chain to adopt the *gt*-conformation (Scheme 71a).¹⁹⁸



Scheme 71: Influence of C5 and C7 configuration on the side chain conformation

The transformation from the predominant *gg*- to *gt*-conformation on going from the natural isomer to the donor **206** arises from a combination of dipolar and steric interactions between the C5-N5 bond and the side chain (Scheme 71a). This resulted in the glycosylation equatorial selectivity, as the donors with *gt*-conformation are less reactive,²¹⁴ and therefore have less chance to form axial glycosides than their *gg*-

isomers.¹⁴⁴ Furthermore, investigations on the influence of configuration at the 7-position with NeuAc donor **66** and 7-*epi*-NeuAc donor **205**¹⁴³ showed that the 7-*epi* sialic acids **205** underwent a conformational change from *gg*- to *gt*- again to avoid unfavorable dipolar and steric interactions with the C5-N5 bond (Scheme 71b). Extrapolating these results, it is predicted that the inversion of both the configurations at C-5 and C-7, as in pseudaminic acid, would again result in unfavorable dipolar and steric repulsion in the hypothetical 5,7-bis-*epi* system **310**, due to which the side chain conformation would change from the predominant *gt* to the most-disarming and the least active *tg*-conformer (Scheme 71c).

Therefore, the trio of inversion of the configurations in Pse with respect to NeuAc, especially the two at C-5 and C-7 would again result in unfavorable dipolar and steric repulsion are expected to cause the Pse donor **311** to adopt the most disarming and the least active *tg*-conformer (Figure 15). Consequently, compared to the moderately equatorial selective Leg donor **181**, an excellent equatorial selectivity in the glycosylation reaction of the Pse donor **311** was expected. Accordingly, the synthesis of the Pse donor **311** and its stereocontrolled equatorial synthesis are the focus of this chapter.

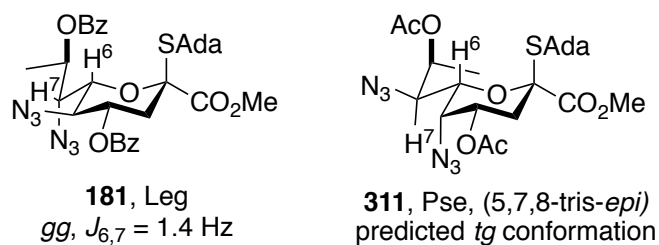


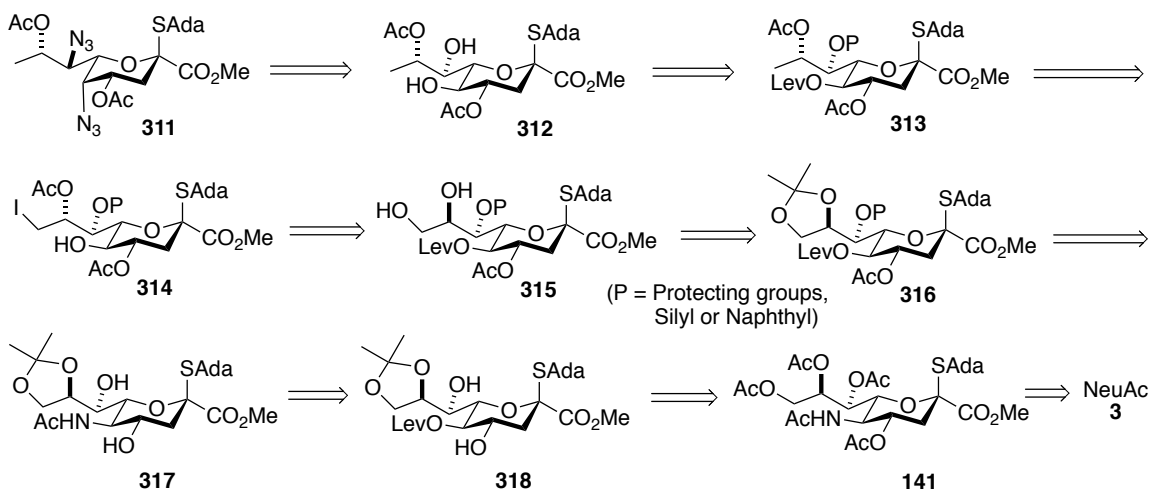
Figure 15: Equatorially selective Leg and Pse donors

4.2 Results and Discussion

4.2.1 Retrosynthesis

Utilizing the strategies developed and lessons learnt from the syntheses of the Leg donor **181**¹⁶² and 5-*epi* NeuAc donor **206**,¹⁹⁸ the synthesis of the Pse donor **311** starting from the readily available NeuAc was planned. The approach for the deoxygenation at the 9-position and replacement of C-O by a C-N bond at the 7-position utilized for the synthesis of the Leg donor **181** (Scheme 37) still remains valid for the synthesis of the Pse donor **311**. However, in addition to this, the preparation of **311** requires inversion of configuration at positions 5 and 7. Similar to Kiefel's work,^{39,140} the synthesis of a 5,7-diol such as **312** was envisioned, which could be suitably activated for displacement by azide anion affording the two C-N bonds at the 5- and 7-positions with inversion of configuration. Deoxygenation at the 9-position can be achieved by the usual means¹⁶² of selective trisylation with TPSCl, then replacement of the -OTPS group by the iodo group as in **314**, and finally dehydroiodination either by radical methods or hydrogenolysis giving **313**. A key step would be the replacement of the equatorial acetamide at the 5-position of **317** by a hydroxyl group with retention of configuration. As was the case for the synthesis of 5-*epi*-NeuAc donor **206**,¹⁹⁸ the Zbiral oxidative deamination^{145,197} was the intended approach, with levulinic acid as nucleophile to give **316**, which facilitates the selective functionalization of the synthesized ester at the 5-position. Utilizing the differential reactivity of the 4- and 7-hydroxy groups of **318**, the 4-hydroxy group can be monoacetylated. Subsequent protection of the 7-hydroxy group with either silyl or naphthyl ethers protecting groups is expected to give **317**. The

substrate **318**, in turn, can be obtained in sequence of known reactions from *N*-acetylneuraminic acid (Scheme 72).^{131,161}



Scheme 72: Retrosynthetic analysis of the proposed Pse donor 311

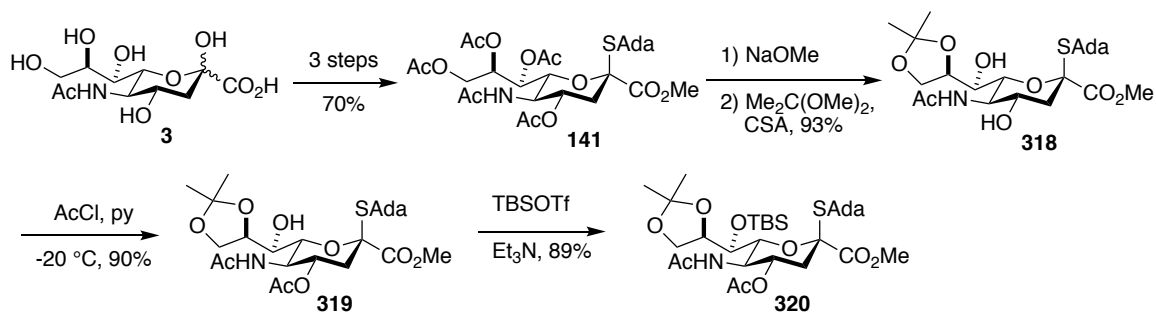
4.2.2 Attempted synthesis of a Pse donor

4.2.2.1 Synthesis of a 7-*O*-TBDMS thioglycoside

The approach of protecting the 7-hydroxy group with a silyl protecting group (OTBDMS) was employed first. Keeping in mind the usually modest yields of the oxidative deamination reactions, the initial plan was to perform this reaction at a late stage of the synthesis.

Thus, NeuAc was converted to the common intermediate, the thioglycoside **141** on a 20 g scale by three well-known literature steps^{131,161} with 70% overall yield. Thereafter, Zemplen deacetylation to remove the acetates and then installation of an 8,9-*O*-acetonide in another known sequence of reactions gave **318** in 93% yield. Then, acetate protection of the 4-OH regioselectively in the presence of the 7-OH following the established pattern¹⁶⁰ gave the monoacetate **319**. This was followed by silylation using TBSOTf to give the 7-*O*-TBDMS protected thioglycoside **320** in 89% yield (Scheme 73).

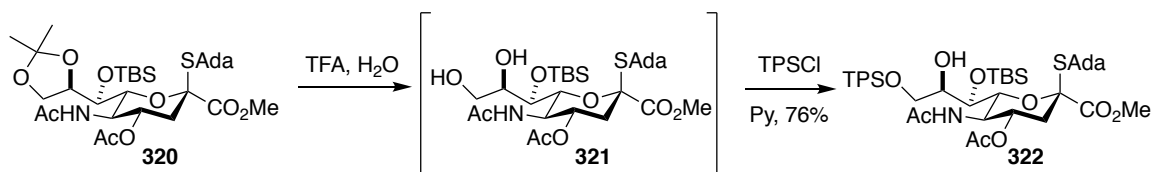
It is noteworthy here that the more common approach of silylation using TBSCl and imidazole mixture did not give the desired product, possibly owing to the hindered nature of the 7-OH group.



Scheme 73: Synthesis of the 7-*O*-TBDMS thioglycoside 320

4.2.2.2 Selective functionalization of 9-hydroxy group

Acid mediated cleavage of the 8,9-*O*-acetonide in **320** gave the intermediate 8,9-diol derivative **321** as a crude product, which was then reacted with 2,4,6-triisopropyl benzenesulfonyl chloride (TPSCI) in the presence of pyridine to give the monotrissylated product **322**, functionalized at the primary 9-OH regioselectively in the presence of the secondary 8-OH (Scheme 74).

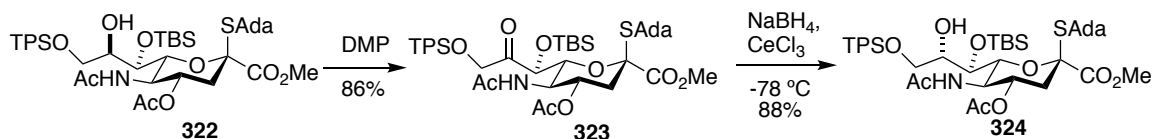


Scheme 74: Synthesis of the 8,9-diol derivative 321 and its selective trisylation

4.2.2.3 Inversion of configuration of a 8-hydroxy group

Oxidation of **322** with Dess-Martin periodinane¹⁶⁷ gave the ketone **323** in 86% yield, which was then subjected to the Luche reduction¹⁷¹ conditions using sodium borohydride and cerium trichloride to give the inverted alcohol **324** as the single isomer in 88% yield (Scheme 75). A similar reaction sequence for the inversion of configuration

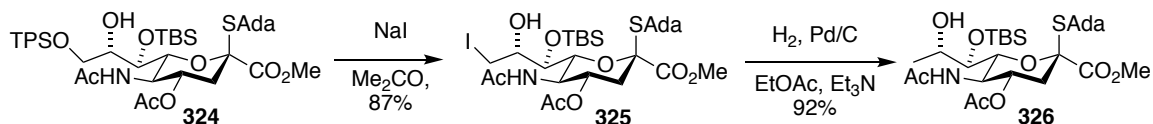
of 7-hydroxy group in Leg synthesis gave a 85:15 mixture of isomers.¹⁶² The steric hindrance arising from the TBDMS group on the vicinal C-7 and also the bulky trisyl group at C-9 presumably assisted the reaction to give the single isomer here in the present case.



Scheme 75: Inversion of configuration at C-8 of 322

4.2.2.4 Synthesis of a 9-deoxy sialoside

The sulfonate ester at the 9-position of **324** was displaced by an iodo group using sodium iodide to give **325** in 87% yield, which was then subjected to hydrogenolysis using a mixture of ethyl acetate and triethylamine to give the 9-deoxy derivative **326** in 92% yield (Scheme 76). In the legionaminic acid series, the same hydrogenolysis reaction albeit without triethylamine had cleaved the thioglycoside moiety to some extent, forcing the adoption of radical reaction route for hydrodeiodination.¹⁶² The use of triethylamine in this reaction essentially solved this problem.

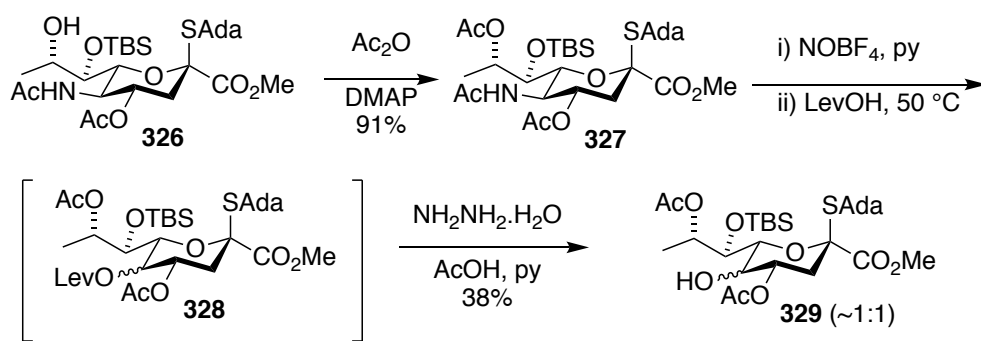


Scheme 76: Synthesis of the 9-deoxy sialoside 326

4.2.2.5 Attempted late stage oxidative deamination with levulinic acid

Compound **326** was reacted with acetic anhydride in the presence of DMAP and triethylamine to give **327**, which was the substrate intended for the late stage oxidative deamination reaction. Reacting **327** with sodium tetrafluoroborate in pyridine gave the corresponding *N*-nitrosoacetamide, which on heating with levulinic acid at 50 °C gave the

levulinyl derivative **328** as the oxidative deamination product in the form of a mixture of diastereomers. It is noteworthy that the synthesized *N*-nitrosoacetamide derivative was quite stable to levulinic acid at lower temperatures under standard conditions, necessitating the adoption of the thermal condition by warming the reaction mixture to 50 °C, similar to that of the Kiefel group's approach.³⁹ In order to facilitate purification of the crude levulinate mixture, **328** was reacted with hydrazine monohydrate in acetic acid and pyridine, which led to the deprotection of the levulinate ester furnishing the 5-hydroxy derivative **329** in 38% overall yield from **326** as approximately 1:1 mixture of stereoisomers (Scheme 77).



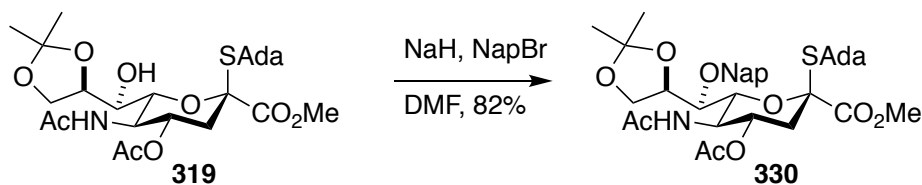
Scheme 77: Attempted late stage oxidative deamination with levulinic acid

Comparing the result with the previous analyses,¹⁵⁹ the poor selectivity in the final deamination reaction is possibly due to the absence of a C-O group at the 9-position, reducing the involvement of the ring oxygen at the 5-position in the stereoretentive participation and supporting more of the carbenium ion-like intermediate. This poor selectivity at such a late stage of the synthesis forced the change of the route, in such a way that the deamination reaction would come before the deoxygenation at the 9-position.

4.2.3 Synthesis of a Pse donor

4.2.3.1 Synthesis of a 7-*O*-naphthyl thioadamantyl sialoside

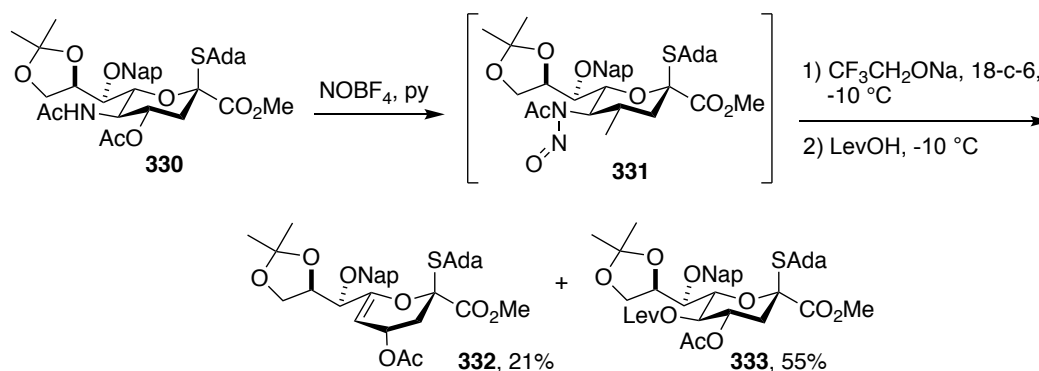
The 7-*O*-naphthylmethyl ether **330** was obtained from the intermediate **318** by treating with sodium hydride and 2-naphthylmethyl bromide²²¹ at 0 °C on a multigram scale in 82% yield, so giving the substrate for the early oxidative deamination (Scheme 78).



Scheme 78: Synthesis of the 7-*O*-naphthyl thioadamantyl sialoside **330**

4.2.3.2 Oxidative deamination with levulinic acid as nucleophile

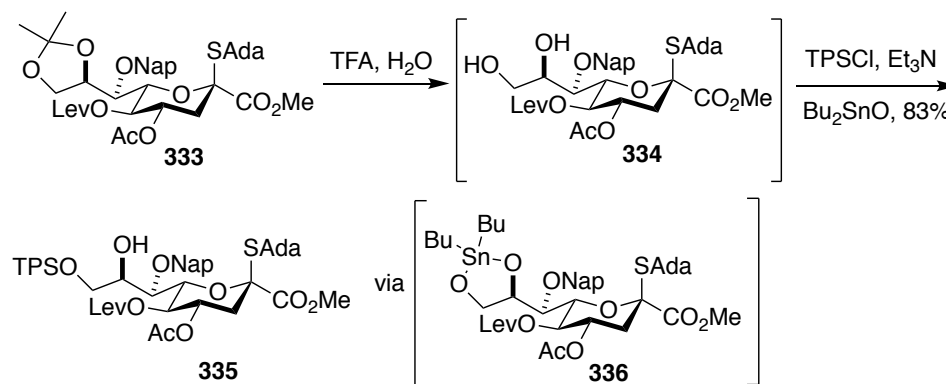
The *N*-nitrosoacetamide derivative **331** was obtained by reacting the substrate **330** with nitrosyl tetrafluoroborate in pyridine at -10 °C, and was then treated with a mixture of preformed sodium trifluoroethoxide and 18-crown-6 in dichloromethane at -10 °C. This was followed by addition of levulinic acid before quenching with sodium bicarbonate at 0 °C. The desired levulinate **333** was obtained in the form of a single equatorial diastereomer, alongside the alkene **332**, a typical byproduct¹⁵⁹ in the oxidative deamination reaction (Scheme 79). The ability to perform this reaction in >5 g batches of **330** enabled scale-up for the total synthesis of Pse, in spite of the modest yield of the reaction.



Scheme 79: Oxidative deamination of 330 with levulinic acid as nucleophile

4.2.3.3 Selective functionalization of the 9-hydroxy group

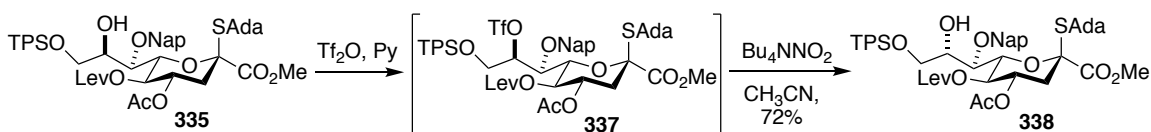
Aqueous trifluoroacetic acid mediated removal of the acetonide gave the corresponding 8,9-diol intermediate **334**. As in the synthesis of the legionaminic acid intermediate **168** and also the 7-OTBDMS containing intermediate **322**, selective functionalization of the primary C-9 hydroxy group was attempted using 2,4,6-triisopropyl benzenesulfonyl chloride (TPSCl) in the usual way.¹⁶² However, presumably due to the presence of a bulky substituents on C-7 and C-5, the reaction time increased substantially (more than 40 h), with the procurement of only 55% of the product **335** and the recovery of 20% of the starting material **334**. Therefore, the procedure was slightly modified by incorporating dibutyltin oxide and replacing pyridine by triethylamine as base in the reaction,^{222,223} to obtain the desired product **335** in 83% yield (Scheme 80). Formation of the dibutyltin acetal derivative **336** in situ presumably assisted this reaction to proceed forward in a shorter time and better yield.



Scheme 80: Selective trisylation of 9-OH via a dibutyltin acetal derivative

4.2.3.4 Inversion of configuration of the 8-hydroxy group

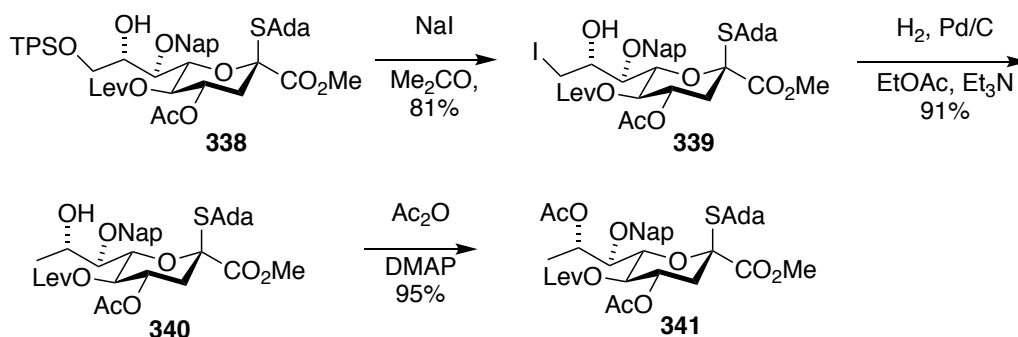
The usual route of oxidation to the ketone and then reduction to obtain the hydroxyl group with inversion of configuration, as were done to obtain **179** and **324**,¹⁶² couldn't be performed here due to the incompatibility with the ketone functionality of the levulinoyl moiety in the reduction step. Therefore, in order to invert the configuration of the C8-hydroxy group, the Lattrell-Dax reaction protocol²²⁴⁻²²⁶ was employed. Accordingly, the C8-hydroxy group of **335** was triflated using triflic anhydride in pyridine to obtain the corresponding triflate derivative **337**, which was then reacted with tetrabutylammonium nitrite to give the desired C-8 epimer **338** in 72% yield (Scheme 81). It is noteworthy that the S_N2 displacement with nitrite ion occurred preferentially on the secondary triflate keeping intact the primary arenesulfonate ester, thus proving that the triflate groups are excellent leaving groups in substitution reactions.²²⁷



Scheme 81: Inversion of configuration at C8 of 335

4.2.3.5 Synthesis of a 9-deoxy sialoside

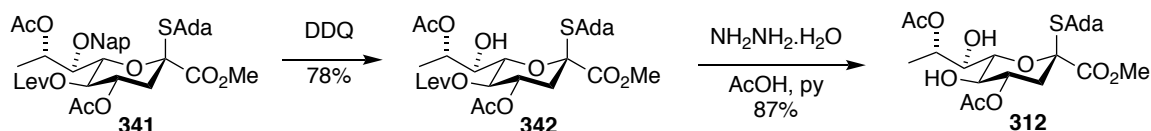
Displacement of the trisyl (-OTPS) group from **338** with sodium iodide under reflux afforded the corresponding iodo derivative **339** in 81% yield. Thereafter, hydrogenolysis over palladium on carbon in the mixture of ethyl acetate and triethylamine, the same protocol that was applied to get **326**, gave the 9-deoxy thioglycoside **340** in 91% yield. Acetylation of **340** with acetic anhydride in the presence of DMAP gave the triester **341** in 95% yield (Scheme 82).



Scheme 82: Synthesis of the 9-deoxy sialoside

4.2.3.6 Deprotection of the 7-naphthyl and 5-levulinoyl groups

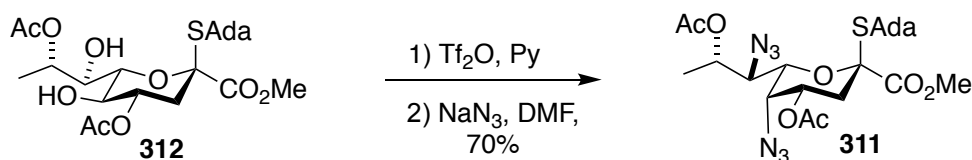
The two hydroxyl protecting groups of **341**, the naphthylmethyl ether at C-7 and the levulinoyl ester at C-5, were then sequentially deprotected using DDQ and hydrazine monohydrate in the usual ways to give **342** and **312**, respectively in good yields (Scheme 83). The access to the diol intermediate **312** was significant, as this was the precursor for the synthesis of the 5,7-diazido Pse donor **311**.



Scheme 83: Deprotection of the naphthyl and levulinoyl groups to access 312

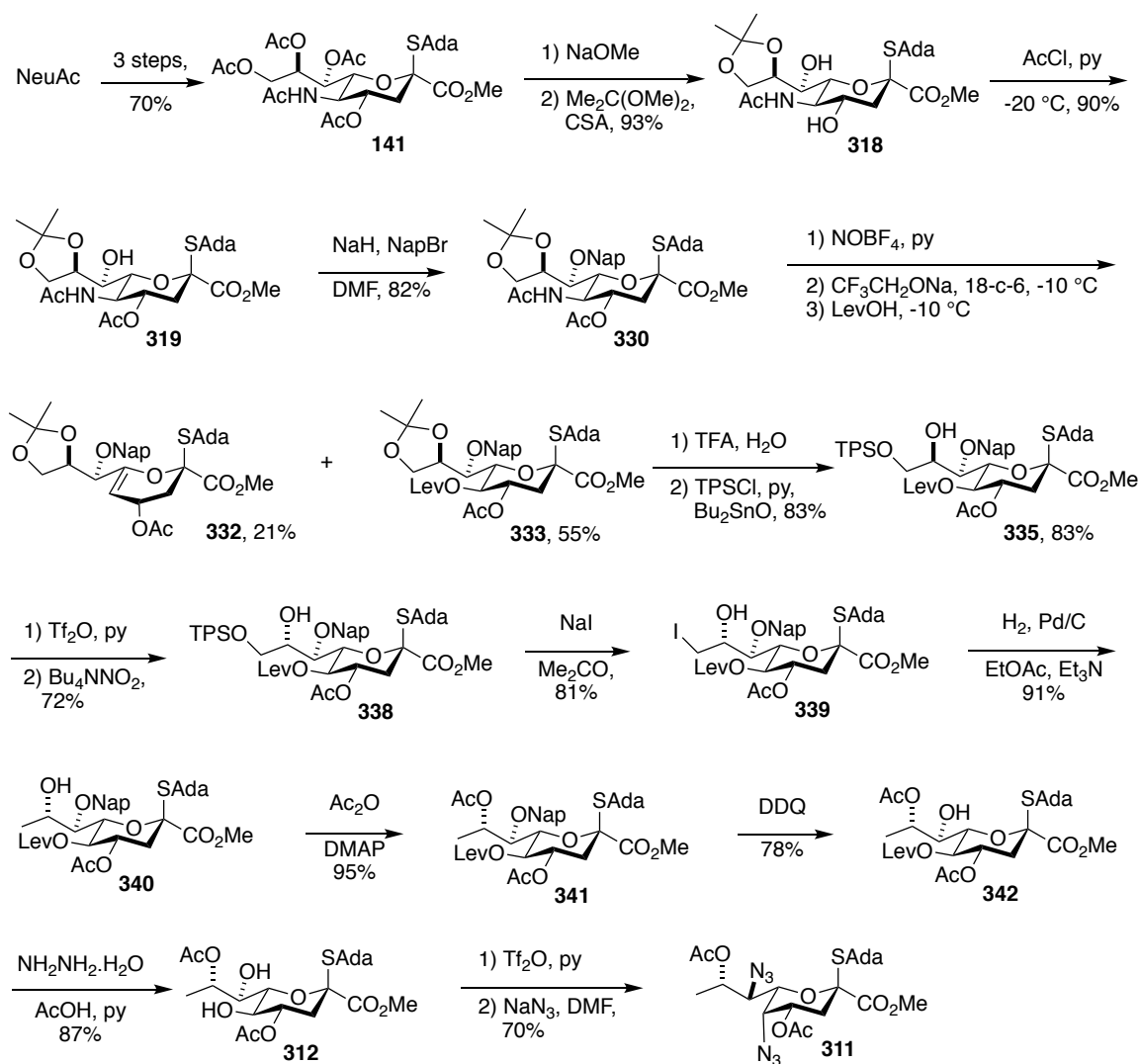
4.2.3.7 Synthesis of the 5,7-diazido Pse donor **311**

The synthesized diol **312** was triflated to give a corresponding ditriflate intermediate, which was then reacted with sodium azide in DMF to give the desired 5,7-diazido Pse donor **311** in 70% yield (Scheme 84). To achieve the Pse donor **311** from the diol **312**, two correct configurations at 5- and 7- positions with C-N bonds were installed in a single sequence of reactions.



Scheme 84: Synthesis of the 5,7-diazido Pse donor **311**

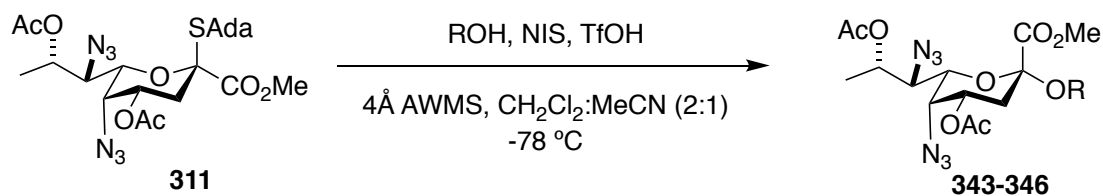
Overall, a practical synthesis of the Pse donor **311** was achieved in 20 steps and 5% overall yield starting from *N*-acetylneuraminic acid. Except for the usual moderate yield of the Zbiral deamination step, all other reactions in the sequence gave good to excellent yields. Therefore, the sequence of reactions described here is simple and scalable, affording the product on the scale of multiple hundreds of milligrams, and offering the opportunity to investigate the glycosylation reaction. The total synthesis of the Pse donor **311** from *N*-acetylneuraminic acid is summarized in Scheme 85.²²⁸



Scheme 85: Total synthesis of the Pse donor 311

4.2.3.8 Glycosylation study with the Pse donor

The synthesized Pse donor **311** was employed in the glycosylation of various acceptors under the same conditions of *N*-iodosuccinimide and triflic acid as the activators in 2:1 mixture of acetonitrile and dichloromethane at -78°C (Scheme 86). These were employed to facilitate comparison with the earlier results obtained with the legionaminic acid donor **181** and the *5-epi*-NeuAc donor **288**.

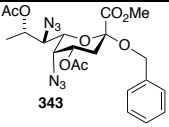
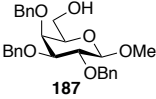
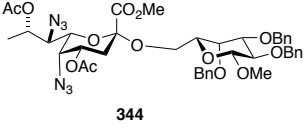
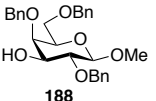
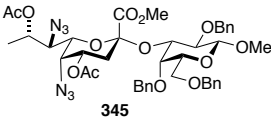
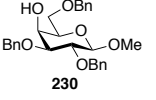
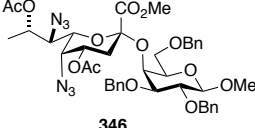


Scheme 86: Glycosylation reactions with the Pse donor 311

The acceptors used were benzyl alcohol, methyl 2,3,4-tri-*O*-benzyl- β -D-galactopyranoside **187**, methyl 2,4,6-tri-*O*-benzyl- β -D-galactopyranoside **188** and methyl 2,3,6-tri-*O*-benzyl- β -D-galactopyranoside **230**. The galactopyranosyl 3- and 6-alcohols **187** and **188** were selected for this study as the linkages obtained from them are the most common ones in the sialic acid glycosides. The highly hindered galactopyranosyl 4-alcohol **230** was selected as a model study, as the linkage Pse-eq.(2 \rightarrow 4)-6-deoxy-*N*-acetylgalactosamide has been found in the repeating unit of the *Pseudomonas aeruginosa* O10 lipopolysaccharides.⁷⁷

Glycosylation of benzyl alcohol gave the equatorial glycoside **343** as a single isomer in 89% yield. In the similar manner, the glycosylation reactions of galactose acceptors, **187** and **188** also afforded the equatorial glycosides **344** and **345**, as single anomers in 83% and 77% yields, respectively. Of particular note is the glycosylation reaction of the highly sterically hindered acceptor **230**, which also afforded **346** with exquisite equatorial selectivity in 53% yield, without formation of the competing axial anomer (Table 10). As usual, the anomeric configuration of **343-346** was determined based on the measurement of the $^3J_{\text{C1-H3axial}}$ heteronuclear coupling constant values, which were in the range of 6.8-7.5 Hz, characteristic of the equatorial glycoside.¹⁷²⁻¹⁷⁵

Table 10: Glycosylation reactions of various acceptors with the Pse donor 311

Entry	Acceptor	Product	Yield	$^3J_{C1,H3}$ (Hz)
1	BnOH	 343	89%	7.1
2	 187	 344	83%	6.8
3	 188	 345	77%	7.5
4	 230	 346	53%	7.3

4.2.3.9 Analysis of side chain conformation and glycosylation selectivity

The glycosylation selectivities obtained with the Pse donor validate the earlier hypothesis, as the selectivities seen here with the same three carbohydrate based acceptors under the same conditions are higher than that obtained with the Leg donor (Table 4, page 51).¹⁶² As expected, analysis of the $^3J_{6,7}$ value of the synthesized donor showed that it has undergone the conformational change from the predominant *gg*- to the least active, *tg*-conformation (Figure 18). This rationalizes the observed glycosylation selectivities, and therefore it can be concluded that the donor side chain conformation is an important aspect in the formation of stereoselective glycosidic bonds.

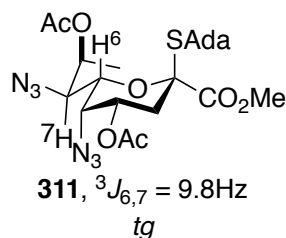


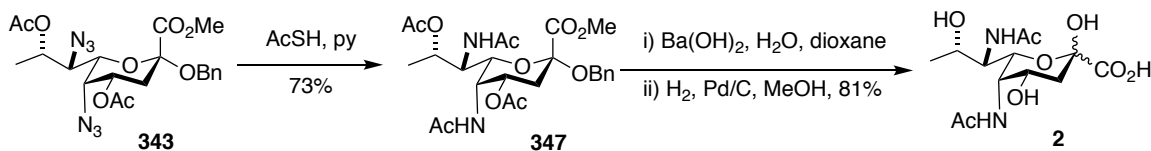
Figure 18: Pse donor 311 in its *tg*-conformation

As far as the Pse donor glycosylation is concerned, the results presented in Table 10 are the first examples of equatorial glycosylation selectivity. Ito *et al.*¹³⁹ performed the glycosylation with the donor **116** under different conditions (acetonitrile at 0 °C), leaving no basis for meaningful comparison. However, the glycosylation reaction with the Pse donor **130** done by Li *et al.*¹⁴¹ under similar conditions of solvent, temperature and activating systems as that for the Pse donor **311** gave mostly unselective or axially selective glycosylated products. Close inspection of Li's donor **130** shows the ${}^3J_{6,7}$ coupling constant value to be 10.0 Hz similar to that of the present donor **311** (9.8 Hz), indicating that both the donors **130** and **311** are in *tg*-conformation about the exocyclic bonds to the side chain. Therefore, the contrast in selectivity between Li's donor **130** and the synthesized Pse donor **311** must arise due to the difference in the amine protecting groups, with the bulkier protecting groups in the Li's donor **130** causing the steric hindrance or hydrogen bonding. Additionally, the bulky isopropyl ester at the anomeric center could have been better accommodated in the equatorial position.

4.2.3.10 Synthesis of the final Pse5Ac7Ac

The glycosylated product **343** obtained from the stereoselective glycosylation of the benzyl alcohol with the Pse donor **311** was treated with excess thioacetic acid in pyridine²²⁹ to afford the corresponding bisacetamide derivative **347** in 73% yield. This was then heated at 60 °C with aqueous barium hydroxide in 1,4-dioxane before

hydrogenolysis over palladium on charcoal also in aqueous 1,4-dioxane afforded the Pse5Ac7Ac **2** in 81% yield (Scheme 87). The spectral data of Pse5Ac7Ac were consistent with those provided by Tsvetkov, Kiefel and others.^{43,97,140,141}



Scheme 87: Deprotection of 343 to access Pse5Ac7Ac 2

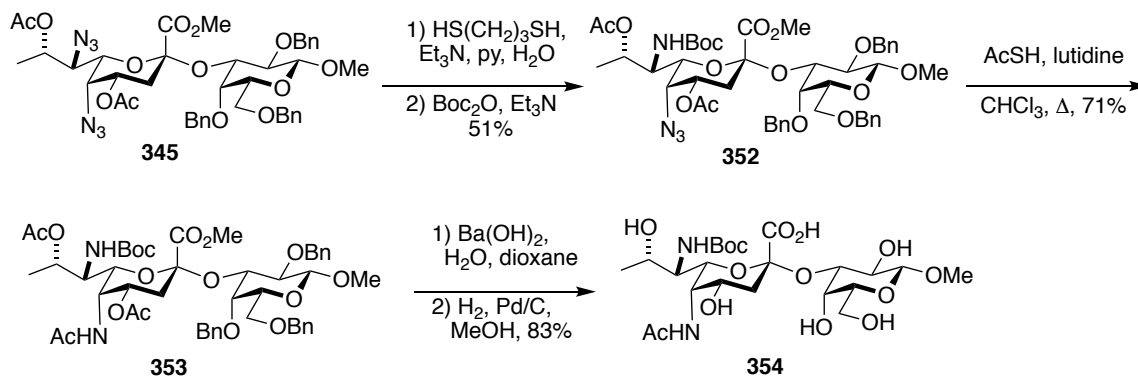
4.2.3.11 Regioselective reduction of azide in the deprotection of 345

Most of the natural Pse glycosides found in various pathogenic bacteria, including the axially-linked *Pseudomonas aeruginosa* 1244 pilin glycoside **30**⁸⁸ and Pse-equatorial (2→4)-6-deoxy-*N*-acetylgalactosaminide linkage formed in the repeating unit of *Pseudomonas aeruginosa* O10 lipopolysaccharide **27**,⁷⁷ possess two different amides at positions 5 and 7. To access the Pse glycosides with differential amide functionalities, two overall routes can be envisaged. This is possible to start by synthesis of a donor with differentially protected amines at positions 5 and 7, as in the synthesis of the Li's donor **130**. Alternatively, the regioselective unmasking of one of the two identically protected amines can be attempted after the glycosylation.

Kiefel *et al.*³⁹ showed in a different substrate having incorrect configuration at the 7-position that the side chain azide was more reactive than the axial azide of the pyranose ring toward PPh₃ mediated Staudinger reaction (Scheme 88).

4.2.3.12 Alternate regioselective reduction of azides in the deprotection of 345

The reversed sequence of first reducing an azide regioselectively into acetamide, followed by conversion of the second azide into a *tert*-butylcarbamate was attempted in the glycoside **345**. Accordingly, the azide at the 7-position was first reduced to the corresponding amine with 1,3-propanedithiol and triethylamine in wet pyridine followed by protection with Boc group to give the corresponding C7 *tert*-butylcarbamate **352**, leaving C5 azide intact. Then, thioacetic acid mediated reduction of the remaining azide at C5 converted it to the corresponding acetamide containing derivative **353**. In the end, global deprotection of all the esters with hot aqueous barium hydroxide in dioxane followed by benzyl deprotection using hydrogenolysis gave the final deprotected product **354** containing a C7 *tert*-butylcarbamate and a C5 acetamide, whereby the 7-position can be further functionalized by cleaving the Boc group (Scheme 90).



Scheme 90: Alternate regioselective reduction of azide in the deprotection of 351

4.3 Conclusion

In conclusion, the synthesis of a diazido based pseudaminic acid donor was achieved in 20 steps and 5% overall yield from the readily available *N*-acetylneuraminic acid. The synthesis is easily scalable to multi-hundred milligram quantities and provided sufficient material for the glycosylation studies. The pseudaminic acid donor turned out

to be an excellent donor, providing the corresponding equatorial glycosides with exquisite selectivity when glycosylated to various primary and secondary alcohols. It was found that the selectivity was the factor of the side chain conformation of the donor, and the tg-conformation enforced on the new donor, owing to its inversion of configuration at 5- and 7-positions in comparison to *N*-acetylneuraminic acid. This study elucidated the role of the side chain conformation in the reactivity and selectivity of the glycosyl donors, which in turn should assist in the mechanism-based development of stereoselective glycosylation reactions. Furthermore, keeping in mind the differentially substituted amine functionality in the bacterial lipopolysaccharides, the regioselective reduction of azide was achieved in the deprotection reactions, leading to differentially substituted amine functionalities in the final deprotected pseudaminic acid glycoside.

CHAPTER 5. CONCLUSION

Legionaminic acid and pseudaminic acid, two rare classes of bacterial sialic acid, are 9-deoxy analogues of the most common sialic acid, *N*-acetylneuraminic acid. These two derivatives are significant given the fact that their glycosides are found in lipopolysaccharides and glycoproteins of several pathogenic Gram-negative bacteria including *Pseudomonas aeruginosa*,⁵² *Legionella pneumophila* serogroup 1,³¹ *Campylobacter jejuni*^{33,34} and *Campylobacter coli*.³⁴ Accordingly, the efficient synthesis of a legionaminic acid and pseudaminic acid donor and their stereoselective glycosylation reactions were the focus of this thesis.

A legionaminic acid donor was synthesized in 15 straightforward steps and 17% overall yield from the commercially available *N*-acetylneuraminic acid. The synthesized donor, when glycosylated to various primary and secondary alcohols including a sialic acid based acceptor, with *N*-iodosuccinimide and triflic acid as activator at -78 °C in 1:2 acetonitrile/dichloromethane, afforded equatorial glycosides in good to excellent yields and selectivities. The less than perfect selectivity with this donor was attributed to the 9-deoxy nature of the side chain, which made it less disarming and afforded greater oxocarbenium-like character in the transition state. A four-step deprotection sequence of the selected glycosides then afforded the final deprotected disaccharides.¹⁶²

An intermediate donor, namely azido 5-*epi*-NeuAc, having the same C-5 configuration as pseudaminic acid was synthesized. Glycosylation reactions of various primary and secondary acceptors with the donor in presence of NIS/TfOH as activator and 1:2 acetonitrile/dichloromethane at -78 °C afforded exclusively equatorial glycosides in good to excellent yields. The synthesis of a 5-azido NeuAc donor followed by

glycosylation reactions of various acceptors with it under the same glycosylation conditions also afforded exquisite equatorial selectivity, in contradiction to the selectivity of the various literature 5-azido NeuAc donors. This led to the conclusion that the low temperature of -78 °C is crucial for the selectivity. The comparable glycosylation selectivity observed in the azido based NeuAc donors with either axial or equatorial azides at the 5-position was attributed to the conformational change of the exocyclic bond enforced by the change in configuration at C5. The selected glycosides were then deprotected in the three-step deprotection sequence to afford the final fully deprotected glycosides.¹⁹⁸

A pseudaminic acid donor was synthesized in 20 steps and 5% overall yield from the commercially available *N*-acetylneuraminic acid. Glycosylation reactions of various primary and hindered secondary alcohols with NIS/TfOH as activators and 1:2 acetonitrile/dichloromethane at -78 °C afforded exquisite equatorial selectivity in good to excellent yields. The excellent selectivity observed was attributed to the inversion of configurations at 5- and 7-positions, which cause the side chain to take up the least reactive *tg*-conformation. Thereafter, regioselective reduction of the azides afforded differential amine functionalities in the final deprotected glycoside, suitable for the further functionalization to access the bacterial lipopolysaccharides.

CHAPTER 6. EXPERIMENTAL SECTION

General experimental: Commercially available starting materials were used without further purification. All solvents were dried according to standard methods. TLC was performed on pre-coated glass plates employing UV absorption and charring with ceric ammonium molybdate (CAM) for visualization. Silica gel 60, 230–400 mesh, 40–63 μm were used for column chromatography. ^1H and ^{13}C NMR spectra were recorded on 400 and 600 MHz instruments at 300 K. Chemical shifts were calibrated to residual solvent peaks. Stereochemical assignments of all coupled glycosides were made with the assistance of $^3J_{\text{C1-H3ax}}$ values.^{143,172-175} Specific rotations were measured using a digital polarimeter and are given with units of $10^{-1} \text{ deg}\cdot\text{cm}^2\cdot\text{g}^{-1}$. High-resolution mass spectra were recorded with an electrospray source coupled to a time-of-flight mass analyzer.

Methyl (1-Adamantanyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero- β -D-galacto-non-2-ulopyranosid)onate (141): To a solution of methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy- β -D-glycero-D-galacto-2-nonulopyranosonate¹⁶¹ (22 g; 41.44 mmol) in anhydrous CH_2Cl_2 (250 mL) was added 1-adamantanethiol (8.1 g; 45.4 mmol) followed by $\text{BF}_3\cdot\text{OEt}_2$ (12.8 mL; 49.7 mmol). The resulting solution was stirred at room temperature under argon for 10 h and then diluted with CH_2Cl_2 (350 mL), washed with saturated aqueous NaHCO_3 , dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was subjected to column chromatography over silica gel eluting with 10% isopropanol in toluene to give **141** as a white solid (21.2 g; 80%), with ^1H and ^{13}C NMR consistent with the reported data.¹³¹ ^1H NMR (400 MHz, CDCl_3) δ 5.72 (d, $J = 10.3$ Hz, 1H), 5.47 (s, 1H), 5.27 (td, $J = 10.9, 3.9$ Hz, 1H), 5.15 (d, $J = 8.2$ Hz, 1H), 5.01 (d, $J = 12.3$ Hz, 3H), 4.55 (d, $J = 11.5$ Hz, 1H),

4.25 – 4.16 (m, 1H), 4.07 (q, $J = 10.0$ Hz, 3H), 3.82 (s, 3H), 2.53 (dd, $J = 13.4, 3.7$ Hz, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.03 – 1.94 (m, 9H), 1.86 (s, 6H), 1.65 (s, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.2, 170.7, 170.2, 170.1, 170.0, 169.7, 85.9, 77.2, 73.9, 72.5, 69.1, 68.8, 63.2, 52.6, 50.3, 49.3, 43.3, 39.7, 35.7, 29.6, 23.0, 20.9, 20.7, 20.5, 20.5.

Methyl (1-adamantanyl 3,5-dideoxy-5-*N*-(1,1-dimethylethoxy)carbonyl-9-*O*-(2,4,6-triisopropylbenzenesulfonyl)-2-thio- β -D-galacto-non-2-

ulopyranosid)onate (168): Compound **162** (0.95 g, 1.78 mmol), obtained by a literature procedure¹³¹ from *N*-acetylneuraminic acid via compound **141** in overall 65% yield as an off-white foam, was dissolved in anhydrous pyridine (15 mL) and 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCI) (3.23 g, 10.68 mmol, 6 eq) was added portion-wise. The mixture was stirred at room temperature for 20 h and the reaction was monitored by TLC (hexane/ethyl acetate 1:1). After completion, the reaction was quenched by addition of methanol (0.43 mL, 10.68 mmol) and the mixture was concentrated *in vacuo* to dryness. The residue was adsorbed on silica gel and purified by flash column chromatography (hexane/ethyl acetate 3:2) to give compound **168** (1.01 g, 71%) as a white foam. $[\alpha]_{\text{D}}^{25} -50.0$ (c 1.5, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.17 (s, 2H), 4.74 (d, $J = 8.5$ Hz, 1H), 4.50 (dd, $J = 10.6, 2.0$ Hz, 1H), 4.23-4.19 (m, 2H), 4.13-4.06 (m, 3H), 4.01 (s, 1H), 3.77 (s, 3H), 3.72 (d, $J = 6.5$ Hz, 1H), 3.61 (t, $J = 8.1$ Hz, 1H), 3.54 (q, $J = 9.5$ Hz, 1H), 2.93-2.86 (m, 2H), 2.63 (d, $J = 4.9$ Hz, 1H), 2.58 (dd, $J = 13.7, 4.3$ Hz, 1H), 1.99-1.90 (m, 6H), 1.86 (d, $J = 11.7$ Hz), 1.81 (t, $J = 12.6$ Hz, 1H), 1.63 (s, 6H), 1.40 (s, 9H), 1.25 (dd, $J = 6.8, 2.6$ Hz); ^{13}C NMR (151 MHz, CDCl_3) δ 170.7, 157.6, 153.9, 150.9, 128.9, 123.8, 86.0, 81.0, 72.2, 71.6, 69.7, 68.7, 67.9, 54.2, 52.7, 50.2, 43.2,

42.9, 36.0, 34.2, 29.8, 29.6, 28.2, 24.7, 24.7, 23.5, 23.5; HRMS (ESI) m/z calcd for: $C_{40}H_{63}NO_{11}S_2Na$, $[M+Na]^+$ 820.3740; found: 820.3735.

Methyl (1-adamantanyl 3,5,9-trideoxy-9-iodo-5-*N*-(1,1-dimethylethoxy)carbonyl-2-thio-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate (170): Compound **168** (0.49 g, 0.62 mmol) was dissolved in anhydrous acetone (4 mL) and sodium iodide (0.93 g, 6.20 mmol, 10 eq) was added to the mixture. The reaction was heated at 40 °C with stirring for 17 h and the progress was monitored by TLC (hexane/ethyl acetate 2:3). After completion, the mixture was directly adsorbed on silica gel and purified by flash column chromatography (hexane/ethyl acetate 2:3) to give compound **170** (0.37 g, 94%) as an off-white foam. $[\alpha]^{25}_D$ -89.1 (c 3.05, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$) δ 4.89 (d, J = 8.6 Hz, 1H,), 4.17 (d, J = 10.2 Hz, 1H), 4.01 (d, J = 7.3 Hz, 1H), 3.99-3.96 (m, 1H), 3.79 (s, 3H), 3.76 (dd, J = 10.2, 1.9 Hz, 1H), 3.64-3.59 (m, 2H), 3.58-3.52 (m, 2H), 3.24 (br s, 1H), 2.56 (dd, J = 13.5, 4.0 Hz, 1H), 2.29 (d, J = 6.0 Hz, 1H), 1.98 (s, 3H), 1.94 (d, J = 11.9 Hz, 3H), 1.91-1.83 (m, 4H), 1.63 (s, 6H), 1.43 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9, 157.4, 86.1, 81.0, 72.1, 72.0, 69.5, 67.7, 54.2, 52.8, 50.2, 43.3, 42.8, 36.0, 29.8, 28.4, 17.0; HRMS (ESI) m/z calcd for: $C_{25}H_{40}INO_8SNa$, $[M+Na]^+$ 664.1417; found: 664.1434.

Methyl (1-adamantanyl 4,8-di-*O*-benzoyl-3,5,9-trideoxy-9-iodo-5-*N*-(1,1-dimethylethoxy)carbonyl-2-thio-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate (171): Compound **170** (0.60 g, 0.94 mmol) was dissolved in anhydrous pyridine (15 mL). The solution was cooled to 0 °C and benzoyl chloride (0.38 mL, 3.27 mmol, 3.5 eq) was added dropwise. The mixture was stirred at 0 °C for 1 h and the reaction was monitored by TLC (hexane/ethyl acetate 3:2). After completion, the mixture was diluted with ethyl

acetate and washed with sat. NaHCO₃, 1 N HCl, brine, dried over Na₂SO₄, filtered, concentrated, co-evaporated with toluene and dried. The crude material was purified by flash column chromatography (hexane/ethyl acetate 78:22) to give **171** (0.72 g, 91%) as a white foam. $[\alpha]_D^{23}$ -25.8 (c 1.8, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.10 (d, *J* = 7.3 Hz, 2H), 8.00 (d, *J* = 7.4 Hz, 2H), 7.60-7.56 (m, 2H), 7.47 (t, *J* = 7.8 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 2H), 5.61 (td, *J* = 11.2, 4.5 Hz, 1H), 4.95 (d, *J* = 8.7 Hz, 1H), 4.93-4.91 (m, 1H), 4.36 (br s, 1H), 4.32 (dd, *J* = 10.4, 1.0 Hz, 1H), 4.26 (dd, *J* = 11.0, 2.4 Hz, 1H), 4.10 (d, *J* = 4.2 Hz, 1H), 3.92 (q, *J* = 9.6 Hz, 1H), 3.85 (dd, *J* = 11.0, 6.4 Hz, 1H), 3.82 (s, 3H), 2.74 (dd, *J* = 13.2, 4.5 Hz, 1H), 2.12 (dd, *J* = 13.2, 12.2 Hz, 1H), 1.93-1.89 (m, 6H), 1.74 (d, *J* = 11.4 Hz, 3H), 1.54 (d, *J* = 12.3 Hz, 3H), 1.46 (d, *J* = 12.0 Hz, 3H), 1.29 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 170.0, 166.6, 165.8, 157.3, 133.4, 133.3, 130.1, 129.9, 129.8, 129.8, 129.3, 128.4, 128.4, 86.0, 81.1, 74.3, 73.7, 70.2, 68.7, 52.8, 52.5, 50.6, 43.3, 40.4, 35.8, 29.7, 28.1, 17.2; HRMS (ESI) *m/z* calcd for: C₃₉H₄₈INO₁₀SNa, [M+Na]⁺ 872.1941; found: 872.1907.

Methyl (1-adamantanyl 5-azido-4,8-di-*O*-benzoyl-3,5,9-trideoxy-2-thio-*D*-glycero-β-*D*-galacto-non-2-ulopyranosid)onate (174): A stirred solution of compound **171** (0.46 g, 0.54 mmol) and tris(trimethylsilyl)silane (0.25 mL, 0.81 mmol, 1.5 eq) in deoxygenated benzene (10 mL) was heated to 60 °C and treated dropwise with a 0.1 M 2,2'-azobis(2-methylpropionitrile) solution in benzene (0.54 mL, 0.1 eq). The reaction was monitored by TLC (hexane/ethyl acetate 3:2). After full consumption of the starting material (1 h), the mixture was cooled down and concentrated *in vacuo* to dryness to give a crude preparation of compound **172**. The residue was dissolved in anhydrous methanol (2.5 mL) and 2 M hydrogen chloride solution in diethyl ether (5 mL) was added. The

mixture was stirred at room temperature for 2 h and then concentrated. The crude residue was dissolved in acetonitrile and the solution was extracted with hexanes to remove any non-polar impurities from the previous step. The acetonitrile layer was separated and concentrated to give a crude preparation of compound **173** that was taken up in a mixture of acetonitrile and water (4:1, 10 mL) and the mixture was cooled to 0 °C. Then triethylamine (0.23 mL, 1.62 mmol, 3 eq), copper (II) sulfate (9 mg, 0.05 mmol, 0.1 eq) and imidazole-1-sulfonyl azide hydrochloride¹⁶⁵ (0.17 g, 0.81 mmol, 1.5 eq) were added to the reaction mixture. The mixture was allowed to warm to room temperature and was stirred for 8 h with reaction progress being monitored by TLC (hexane/ethyl acetate 3:2). After full consumption of the starting material, the mixture was diluted with ethyl acetate and was washed with 1 N HCl. The aqueous phase was then washed with ethyl acetate and combined organic layers were dried over Na₂SO₄, filtered, concentrated and dried. The crude material was purified by flash column chromatography (hexane/ethyl acetate 7:3) to give **174** (0.28 g, 78% over 3 steps) as a white foam. $[\alpha]_D^{23}$ -49.0 (c 1.6, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.05-8.01 (m, 4H), 7.59-7.54 (m, 2H), 7.48-7.42 (m, 4H), 5.65 (ddd, *J* = 11.7, 10.0, 4.7 Hz, 1H), 4.99 (dq, *J* = 8.6, 6.1 Hz, 1H), 4.34 (d, *J* = 10.1 Hz, 1H), 4.03 (d, *J* = 8.6 Hz, 1H), 3.91 (t, *J* = 10.1 Hz, 1H), 3.77 (s, 3H), 2.83 (dd, *J* = 13.4, 4.7 Hz, 1H), 2.77 (br s, 1H), 1.91 (dd, *J* = 13.4, 11.7 Hz, 1H), 1.84 (s, 3H), 1.76 (dd, *J* = 11.7, 1.5 Hz, 3H), 1.62 (dd, *J* = 11.7, 1.2 Hz, 3H), 1.59 (d, *J* = 6.1 Hz, 3H), 1.50 (d, *J* = 12.4 Hz, 3H), 1.36 (d, *J* = 11.8 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.3, 165.4, 165.2, 133.4, 133.1, 130.2, 129.7, 129.6, 129.3, 128.5, 128.5, 85.6, 72.3, 71.2, 71.1, 70.5, 60.8, 52.8, 50.3, 43.2, 39.7, 35.7, 29.6, 17.2; HRMS (ESI) *m/z* calcd for: C₃₄H₃₉N₃O₈SNa, [M+Na]⁺ 672.2356; found: 672.2335.

Methyl (1-adamantanyl 5-azido-4,8-di-O-benzoyl-3,5,9-trideoxy-7-oxo-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (177): Compound **174** (0.11 g, 0.17 mmol) was dissolved in anhydrous dichloromethane (2 mL) and the solution was cooled to 0 °C and Dess-Martin periodinane (108 mg, 0.26 mmol, 1.5 eq) was added. The mixture was stirred at room temperature for 4 h and the reaction was monitored by TLC (hexane/ethyl acetate 85:15). After completion, the mixture was diluted with diethyl ether and 20% aqueous Na₂S₂O₃ solution was added. The organic layer was then washed with brine, dried over Na₂SO₄, filtered, concentrated and dried. The crude material was purified by flash column chromatography (hexane/ethyl acetate 9:1) to give **177** (0.094 g, 85%) as a white foam. $[\alpha]_D^{25}$ -76.2 (c 2.05, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.09-8.07 (m, 2H), 8.02-8.00 (m, 2H), 7.58-7.55 (m, 2H), 7.46-7.42 (m, 4H), 5.57 (q, *J* = 6.8 Hz, 1H), 5.47 (ddd, *J* = 11.2, 9.7, 4.8 Hz, 1H), 5.03 (d, *J* = 9.8 Hz, 1H), 3.84 (t, *J* = 9.8 Hz, 1H), 3.79 (s, 3H), 2.80 (dd, *J* = 13.7, 4.8 Hz, 1H), 2.03 (d, *J* = 11.7 Hz, 3H), 1.99-1.94 (m, 4H), 1.91 (d, *J* = 11.7 Hz, 3H), 1.70 (d, *J* = 6.8 Hz, 3H), 1.63 (s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 202.6, 169.8, 166.0, 165.2, 133.4, 133.4, 129.9, 129.7, 129.3, 129.1, 128.5, 128.4, 86.2, 73.7, 73.5, 70.6, 60.9, 52.8, 50.7, 43.3, 39.1, 35.9, 29.8, 16.2; HRMS (ESI) *m/z* calcd for: C₃₄H₃₇N₃O₈SNa, [M+Na]⁺ 670.2199; found: 670.2187.

Methyl (1-adamantanyl 5-azido-4,8-di-O-benzoyl-3,5,9-trideoxy-2-thio-D-glycero-β-L-altro-non-2-ulopyranosid)onate (179): A solution of compound **177** (0.172 g, 0.265 mmol) in dichloromethane (2.25 mL) was cooled to -78 °C and a solution of cerium(III) chloride heptahydrate (0.30 g, 0.79 mmol, 3 eq) in methanol (4.75 mL) was added. After 1 h sodium borohydride (0.015 g, 0.398 mmol) was added and the reaction was monitored by TLC (hexane/ethyl acetate 7:3). After completion, the mixture was

quenched with sat aqueous NH_4Cl , warmed to room temperature and concentrated. The residue was diluted with ethyl acetate, washed with water and brine dried over Na_2SO_4 , filtered, concentrated and dried. The crude material was purified by flash column chromatography (hexane/ethyl acetate 3:1) to give **179** (0.14 g, 82%) as a white foam and the recovered substrate **174** (0.24 g; 14%). $[\alpha]_{\text{D}}^{22}$ -68.4 (c 3.5, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 8.06 (dd, $J = 8.2, 1.1$ Hz, 2H), 8.01 (dd, $J = 8.2, 1.1$ Hz, 2H), 7.58-7.53 (m, 2H), 7.45-7.42 (m, 4H), 5.57 (ddd, $J = 11.2, 9.4, 4.8$ Hz, 1H), 5.53 (dt, $J = 12.3, 6.3$ Hz, 1H), 4.38 (dd, $J = 10.2, 2.9$ Hz, 1H), 4.06 (t, $J = 10.2$, 1H), 4.04 (dd, $J = 12.3, 2.9$, 1H), 3.57 (s, 3H), 2.97 (br s, 1H), 2.72 (dd, $J = 13.6, 4.8$ Hz, 1H), 1.99-1.96 (m, 6H), 1.94 (dd, $J = 13.6, 11.2$ Hz, 1H), 1.83 (d, $J = 10.3$ Hz, 3H), 1.64-1.59 (m, 6H), 1.49 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.2, 166.0, 165.2, 133.4, 133.0, 130.4, 129.7, 129.7, 129.3, 128.5, 128.3, 85.7, 75.2, 71.9, 71.8, 70.9, 60.3, 52.5, 50.4, 43.4, 39.1, 35.9, 29.8, 17.4; HRMS (ESI) m/z calcd for: $\text{C}_{34}\text{H}_{39}\text{N}_3\text{O}_8\text{SNa}$, $[\text{M}+\text{Na}]^+$ 672.2356; found: 672.2347.

Methyl (1-adamantanyl 5,7-diazido-4,8-di-O-benzoyl-3,5,7,9-tetra-deoxy-2-thio-D-glycero- β -D-galacto-non-2-ulopyranosid)onate (181): Compound **179** (0.14 g, 0.21 mmol) was dissolved in anhydrous dichloromethane (2 mL), the solution was cooled to 0 °C and pyridine (170 μL , 2.10 mmol, 10 eq) was added followed by a drop-wise addition of triflic anhydride (106 μL , 0.63 mmol, 3 eq). The mixture was stirred at 0 °C for 1 h and the reaction was monitored by TLC (hexane/ethyl acetate 7:3). After completion, the mixture was diluted with dichloromethane and poured into ice-cold 1 N HCl solution. The organic phase was washed with cold water, dried over Na_2SO_4 , filtered and concentrated to get a crude preparation of **180**. The crude **180** was dissolved in anhydrous

dimethylformamide (4 mL) and the solution was cooled to 0 °C followed by addition of sodium azide (0.27 g, 4.20 mmol, 20 eq). The mixture was stirred at 0 °C for 16 h and the reaction was monitored by TLC (hexane/ethyl acetate 4:1). After completion, the mixture was diluted with ethyl acetate and washed with water. The organic phase was washed with brine, dried over Na₂SO₄, filtered, concentrated and dried. The crude material was purified by flash column chromatography (hexane/ethyl acetate 93:7) to give **181** (0.12 g, 81% over the two steps) as a white foam. $[\alpha]_D^{21}$ -50.7 (c 2.9, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.06-8.02 (m, 4H), 7.59-7.56 (m, 2H), 7.47-7.43 (m, 4H), 5.65 (ddd, *J* = 11.7, 10.0, 4.7 Hz, 1H), 5.38 (quintet, *J* = 6.2 Hz, 1H), 4.47 (dd, *J* = 10.1, 1.4 Hz, 1H), 3.87 (t, *J* = 10.0 Hz, 1H), 3.85 (dd, *J* = 6.4, 1.4 Hz, 1H), 3.77 (s, 3H), 2.87 (dd, *J* = 13.4, 4.7 Hz, 1H), 1.98 (dd, *J* = 13.4, 11.7 Hz, 1H), 1.89-1.85 (m, 6H), 1.71 (d, *J* = 11.3 Hz, 3H), 1.68 (d, *J* = 6.2 Hz, 3H), 1.55 (d, *J* = 12.2 Hz, 3H), 1.44 (d, *J* = 12.2 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 169.6, 165.3, 165.2, 133.5, 133.3, 129.8, 129.7, 129.7, 129.2, 128.5, 128.5, 85.8, 71.2, 71.0, 71.0, 64.1, 61.5, 52.7, 50.5, 43.2, 39.8, 35.8, 29.6, 17.2; HRMS (ESI) *m/z* calcd for: C₃₄H₃₈N₆O₇SNa, [M+Na]⁺ 697.2420; found: 697.2410.

Methyl (1-adamantanyl 5-azido-3,5,9-trideoxy-2-thio-D-glycero-β-D-galacto-non-2-uloypyranosid)onate (175): Compound **170** (0.35 g, 0.55 mmol) was dissolved in methanol (15 mL) and Pearlman's catalyst (1.55 g, 2.20 mmol) was added to the mixture. The flask was filled with hydrogen gas (45 psi) and the mixture was stirred at room temperature for 48 h until starting material was no longer present. The mixture was filtered through a pad of Celite and the filtrate was concentrated. The residue was dissolved in anhydrous methanol (7.5 mL) and 2 M hydrogen chloride solution in diethyl ether (5 mL) was added. The mixture was stirred at room temperature for 2 h and then

concentrated. The residue was dissolved in a mixture of methanol and water (2:1, 6 mL). The mixture was cooled to 0 °C and potassium carbonate (0.23 g, 1.65 mmol, 3 eq), copper (II) sulfate (9 mg, 0.06 mmol, 0.1 eq) and imidazole-1-sulfonyl azide hydrochloride¹⁶⁵ (0.19 g, 1.10 mmol, 2 eq) were added. The mixture was allowed to warm to room temperature and was stirred for 16 h with reaction progress being monitored by TLC. After consumption of the starting material, the mixture was slightly concentrated, acidified with 1 N HCl and diluted with ethyl acetate. The aqueous phase was then washed with ethyl acetate and the combined organic layers were dried over Na₂SO₄, filtered, concentrated and dried. The crude material was purified by flash column chromatography to give **175** (0.09 g, 39% over the three steps) as a slightly yellow oil. $[\alpha]_D^{22}$ -185.5 (c 4.0, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 4.22 (d, *J* = 10.4 Hz, 1H), 4.03 (ddd, *J* = 11.9, 9.6, 4.7 Hz, 1H), 3.81 (s, 3H), 3.80-3.76 (m, 1H), 3.42 (d, *J* = 8.9 Hz, 1H), 3.34 (t, *J* = 10.0 Hz, 1H), 2.42 (dd, *J* = 13.6, 4.8 Hz, 1H), 1.98-1.96 (m, 6H), 1.92 (d, *J* = 11.0 Hz, 3H), 1.74 (dd, *J* = 13.6, 11.9 Hz, 1H), 1.68 (s, 6H), 1.29 (d, *J* = 6.2 Hz, 3H); ¹³C NMR (150 MHz, CD₃OD) δ 172.4, 85.9, 74.2, 70.8, 67.9, 65.5, 64.1, 52.0, 49.5, 43.0, 42.6, 35.7, 29.9, 19.8; HRMS (ESI) *m/z* calcd for: C₂₀H₃₁N₃O₆SNa, [M+Na]⁺ 464.1831; found: 464.1836.

Methyl (1-adamantanyl 5-azido-7,8-di-*O*-benzoyl-3,5,9-trideoxy-2-thio-*D*-glycero-β-*D*-galacto-non-2-ulopyranosid)onate (176): Compound **175** (0.08 g, 0.18 mmol) was dissolved in anhydrous pyridine (3 mL). The solution was cooled to 0 °C and benzoyl chloride (52 μL, 0.45 mmol, 2.5 eq) was added dropwise. The mixture was stirred at 0 °C for 1 h and the reaction was monitored by TLC. After completion, the mixture was diluted with dichloromethane and washed with sat. NaHCO₃. The aqueous layer was

extracted with dichloromethane and the combined organic layers were washed with water, brine, dried and concentrated. The crude material was purified by flash column chromatography to give **176** (0.05 g, 46%) as a slightly yellow oil. $[\alpha]_D^{23}$ -27.2 (c 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.15 (dd, *J* = 8.2, 1.0 Hz, 2H), 8.02 (dd, *J* = 8.1, 0.9 Hz, 2H), 7.61-7.58 (m, 1H), 7.55-7.53 (m, 1H), 7.49-7.46 (m, 2H), 7.44-7.41 (m, 2H), 5.88 (dd, *J* = 6.0, 1.6 Hz, 1H), 5.43 (quintet, *J* = 6.0 Hz, 1H), 4.36 (dd, *J* = 9.8, 1.6 Hz, 1H), 4.23 (ddd, *J* = 11.8, 9.8, 4.7 Hz, 1H), 3.81 (s, 3H), 3.07 (t, *J* = 9.8 Hz, 1H), 2.52 (dd, *J* = 13.7, 4.7 Hz, 1H), 1.91-1.87 (m, 6H), 1.77-1.72 (m, 4H), 1.58-1.54 (m, 6H), 1.47 (d, *J* = 11.9 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.2, 165.7, 165.6, 133.5, 133.1, 130.1, 130.1, 129.7, 129.4, 128.6, 128.4, 86.0, 72.8, 71.1, 70.9, 68.6, 64.2, 52.6, 50.3, 43.3, 42.5, 35.8, 29.7, 16.1; HRMS (ESI) *m/z* calcd for: C₃₄H₃₉N₃O₈SNa, [M+Na]⁺ 672.2356; found: 672.2387.

Methyl (methyl 5-azido-3,5-dideoxy-D-glycero-D-galacto-non-2-ulopyranosid)onate

(22): Methyl (methyl per-*O*-acetyl-*N*-acetyl-β-D-glycero-D-galacto-non-2-ulopyranosid)onate¹⁵⁹ (5.05 g, 10 mmol) was dissolved in anhydrous THF (40 mL) and di-*tert*-butyl dicarbonate (23.0 mL, 100 mmol, 10 eq) and 4-(dimethylamino)pyridine (0.49 g, 4 mmol, 0.4 eq) were added to the mixture. The reaction mixture was heated to 60 °C with stirring for 22 h and the progress was monitored by TLC (hexane/ethyl acetate 3:2). After completion, the mixture was directly adsorbed on silica gel and purified by flash column chromatography (hexane/ethyl acetate 65:35) to give the *N*-acetyl-*N*-imide as an orange oil. The residue was dissolved in anhydrous methanol (65 mL) and a catalytic amount of sodium methoxide (0.27 g, 5 mmol, 0.5 eq) was added to the mixture. The mixture was stirred at room temperature for 3 h and then 2 M hydrogen chloride

solution in diethyl ether (35 mL) was added. The mixture was stirred at room temperature for 5 h and then concentrated to give the free amine. The residue was dissolved in a mixture of acetonitrile and water (4:1, 150 mL), cooled to 0 °C and triethylamine (4.18 mL, 30 mmol, 3 eq), copper(II) sulfate (0.16 g, 1 mmol, 0.1 eq) and imidazole-1-sulfonyl azide hydrochloride¹⁶⁵ (3.14 g, 15 mmol, 1.5 eq) were added. The mixture was allowed to warm to room temperature and was stirred for 15 h with reaction progress being monitored by TLC (chloroform/methanol 85:15). After full consumption of the starting material, the mixture was diluted with ethyl acetate and was washed with 1 N HCl. The aqueous phase was then washed with ethyl acetate and the combined organic layers were dried over Na₂SO₄, filtered, concentrated and dried. The crude material was purified by flash column chromatography (chloroform/methanol 9:1) to give **182** (0.98 g, 30% over the four steps) as a colorless oil. $[\alpha]_D^{22}$ -61.1 (c 4.85, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 3.97 (ddd, *J* = 11.3, 9.9, 5.0 Hz, 1H), 3.82 (dd, *J* = 11.3, 2.3 Hz, 1H), 3.80 (s, 3H), 3.76-3.73 (m, 1H), 3.73-3.70 (m, 2H), 3.66 (dd, *J* = 11.3, 5.0 Hz, 1H), 3.44 (t, *J* = 9.9 Hz, 1H), 3.22 (s, 3H), 2.28 (dd, *J* = 12.9, 5.0 Hz, 1H), 1.61 (dd, *J* = 12.9, 11.3 Hz, 1H); ¹³C NMR (151 MHz, CD₃OD) δ 169.8, 99.0, 70.5, 70.2, 69.0, 68.0, 63.8, 63.2, 52.0, 50.3, 39.9; HRMS (ESI) *m/z* calcd for: C₁₁H₁₉N₃O₈Na, [M+Na]⁺ 344.1070; found: 344.1067.

Methyl (methyl 5-azido-3,5-dideoxy-8,9-*O*-isopropylidene-*D*-glycero-*D*-galacto-non-2-ulopyranosid)onate (183): Compound **182** (0.88 g, 2.75 mmol) was dissolved in anhydrous acetone (30 mL) and 2,2-dimethoxypropane (0.56 mL, 4.54 mmol, 1.65 eq) was added to the mixture followed by camphorsulfonic acid (22 mg, 0.1 mmol, 0.04 eq). The reaction was stirred at room temperature for 6 h and the progress was monitored by

TLC (toluene/*i*-PrOH 9:1). Upon completion, the reaction was quenched by addition of triethylamine (0.2 mL). After 15 min the reaction mixture was concentrated and the residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was dried over Na₂SO₄, concentrated and purified by flash column chromatography (toluene/*i*-PrOH 95:5) to give **183** (0.82 g, 83%) as a white foam. $[\alpha]_D^{22}$ -59.0 (c 4.2, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 4.14 (dt, *J* = 8.4, 5.4 Hz, 1H), 4.12-4.06 (m, 2H), 4.02 (dd, *J* = 8.4, 4.9 Hz, 1H), 3.96 (d, *J* = 3.5 Hz, 1H), 3.81 (s, 3H), 3.71-3.68 (m, 1H), 3.62 (d, *J* = 10.4 Hz, 2H, H-6, 7-OH), 3.48 (t, *J* = 10.0 Hz, 1H), 3.19 (s, 3H), 2.35 (dd, *J* = 12.9, 5.1 Hz, 1H), 1.63 (dd, *J* = 12.9, 11.6 Hz, 1H), 1.37 (s, 3H), 1.26 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 169.7, 109.3, 99.1, 74.5, 70.7, 70.7, 68.3, 67.7, 62.8, 53.1, 51.2, 39.9, 26.9, 25.3; HRMS (ESI) *m/z* calcd for: C₁₄H₂₃N₃O₈Na, [M+Na]⁺ 384.1383; found: 384.1389.

Methyl (methyl 5-azido-4-*O*-(*tert*-butyldimethylsilyl)-3,5-dideoxy-8,9-*O*-isopropylidene-*D*-glycero-*D*-galacto-non-2-ulopyranosid)onate (184): Compound **183** (0.87 g, 2.4 mmol) and imidazole (0.36 mg, 5.28 mmol, 2.2 eq) were dissolved with stirring in anhydrous DMF (5 mL), the mixture was cooled to 0 °C and *tert*-butyldimethylsilyl chloride (0.40 mg, 2.64 mmol, 1.1 eq) was added. The mixture was allowed to warm to room temperature and the reaction progress was monitored by TLC (hexane/ethyl acetate 3:2). After 20 h DMF was removed *in vacuo* and the residue was dissolved in water and extracted with Et₂O. The combined organic layers were dried over Na₂SO₄, concentrated and purified by flash column chromatography (hexane/ethyl acetate 7:3) to give **184** (1.04 g, 91%) as a white solid. $[\alpha]_D^{22}$ -57.8 (c 2.25, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 4.13-4.09 (m, 2H), 4.06-4.01 (m, 2H), 3.79-3.75 (m, 4H),

3.59 (d, $J = 10.2$ Hz, 1H), 3.47 (t, $J = 10.2$ Hz, 1H), 3.20 (s, 3H), 2.48 (d, $J = 10.3$ Hz, 1H), 2.23 (dd, $J = 13.1, 5.1$ Hz, 1H), 1.65 (dd, $J = 13.1, 11.1$ Hz, 1H), 1.39 (s, 3H), 1.28 (s, 3H), 0.88 (s, 9H), 0.15 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 168.5, 109.2, 99.1, 74.9, 70.7, 70.5, 69.3, 67.5, 63.6, 52.7, 51.0, 40.7, 26.9, 25.6, 25.4, 17.8, -4.6, -5.1; HRMS (ESI) m/z calcd for: $\text{C}_{20}\text{H}_{37}\text{N}_3\text{O}_8\text{SiNa}$, $[\text{M}+\text{Na}]^+$ 498.2248; found: 498.2254.

Methyl (methyl 7-*O*-acetyl-5-azido-4-*O*-(*tert*-butyldimethylsilyl)-3,5-dideoxy-8,9-*O*-isopropylidene-*D*-glycero-*D*-galacto-non-2-ulopyranosid)onate (185): Compound **184** (1.04 g, 2.18 mmol) was dissolved in anhydrous DCM (10 mL), the mixture was cooled to 0 °C and pyridine (3.53 mL, 44 mmol, 20 eq), acetic anhydride (2.06 mL, 22 mmol, 10 eq) and a catalytic amount of DMAP were added with stirring. The reaction mixture was allowed to warm to room temperature and the progress was monitored by TLC (hexane/ethyl acetate 4:1). After 7 h the mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (hexane/ethyl acetate 85:15) to give **185** (1.11 g, 98%) as a colorless oil. $[\alpha]_{\text{D}}^{20}$ -22.1 (c 2.25, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 5.39 (dd, $J = 7.3, 1.5$ Hz, 1H), 4.32 (dt, $J = 7.3, 6.1$ Hz, 1H), 4.04-4.01 (m, 1H), 4.01-3.98 (m, 1H), 3.87 (dd, $J = 8.7, 6.1$ Hz, 1H), 3.79 (s, 3H), 3.62 (dd, $J = 10.6, 1.5$ Hz, 1H), 3.21 (s, 3H), 3.01 (dd, $J = 10.6, 9.1$ Hz, 1H), 2.24 (dd, $J = 13.2, 5.1$ Hz, 1H), 2.17 (s, 3H), 1.66 (dd, $J = 13.1, 11.1$ Hz, 1H), 1.37 (s, 3H), 1.29 (s, 3H), 0.88 (s, 9H), 0.14 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.0, 168.0, 109.2, 99.0, 73.7, 70.8, 70.1, 69.5, 66.6, 64.2, 52.6, 51.1, 40.5, 26.7, 25.6, 25.5, 20.9, 17.8, -4.7, -5.1; HRMS (ESI) m/z calcd for: $\text{C}_{22}\text{H}_{39}\text{N}_3\text{O}_9\text{SiNa}$, $[\text{M}+\text{Na}]^+$ 540.2353; found: 540.2354.

Methyl (methyl 7-*O*-acetyl-5-azido-3,5-dideoxy-8,9-*O*-isopropylidene-*D*-glycero-*D*-galacto-non-2-ulopyranosid)onate (186): Compound **185** (1.11 g, 2.14 mmol) was dissolved in anhydrous THF (25 mL) and 1.0 M tetrabutylammonium fluoride solution in THF (4.28 mL, 2 eq) was added. When TLC (hexane/ethyl acetate 1:1) showed complete conversion, the volatiles were removed *in vacuo* and the residue was purified by flash column chromatography (hexane/ethyl acetate 3:2) to give **186** (0.85 g, 98%) as a colorless oil. $[\alpha]_D^{21}$ -27.5 (c 1.65, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 5.37 (dd, *J* = 7.2, 1.6 Hz, 1H), 4.33 (dt, *J* = 7.2, 6.1 Hz, 1H), 4.08 (ddd, *J* = 11.3, 9.4, 5.1 Hz, 1H), 4.03 (dd, *J* = 8.7, 6.1 Hz, 1H), 3.88 (dd, *J* = 8.7, 6.1 Hz, 1H), 3.79 (s, 3H), 3.66 (dd, *J* = 10.5, 1.6 Hz, 1H), 3.22 (s, 3H), 3.07 (dd, *J* = 10.5, 9.4 Hz, 1H), 2.55 (br s, 1H), 2.38 (dd, *J* = 13.1, 5.1 Hz, 1H), 2.16 (s, 3H), 1.70 (dd, *J* = 13.1, 11.3 Hz, 1H), 1.37 (s, 3H), 1.29 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.1, 167.9, 109.2, 98.8, 73.7, 70.6, 70.0, 68.8, 66.6, 63.4, 52.7, 51.2, 39.6, 26.6, 25.5, 20.8; HRMS (ESI) *m/z* calcd for: C₁₆H₂₅N₃O₉Na, [M+Na]⁺ 426.1488; found: 426.1494.

Acid-washed Molecular Sieves: Molecular sieves (4 Å, 30 g) were soaked in 2 N HCl (200 mL) for 12 h, then filtered and washed with de-ionized water (300 mL). The resulting solid was dried at 254 °C under vacuum for 24 h to give acid-washed molecular sieves (23 g), which were used directly for glycosylation.

General protocol for glycosylation (GP) for donor 181: A mixture of donor **181** (101 mg, 0.15 mmol), acceptor (0.18 mmol, 1.2 eq) and activated 4Å acid-washed molecular sieves (300 mg) in anhydrous dichloromethane/acetonitrile (2:1) (3 mL) was stirred for 2 h at room temperature. Then the mixture was cooled to -78 °C and was treated with *N*-iodosuccinimide (41 mg, 0.18 mmol, 1.2 eq) and trifluoromethanesulfonic acid (2 μL,

0.023 mmol, 0.15 eq). The reaction mixture was stirred at -78 °C until completion and then quenched with triethylamine (25 μ L). The mixture was diluted with dichloromethane, filtered through Celite, washed with 20% aqueous Na₂S₂O₃, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was adsorbed on silica gel and was purified by flash column chromatography to give the desired glycosylation products.

Methyl [1-benzyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetra-deoxy-D-glycero- α -D-galacto-non-2-ulopyranosid)onate] (30): Glycosylation of benzyl alcohol (8.28 μ L, 0.18 mmol, 1.2 eq) with **181** (100 mg, 0.15 mmol) was performed according to general procedure **GP** at -78 °C for 6 h to afford after flash column chromatography (hexane/ethyl acetate 80:20) compound **191** as the only product as colorless oil (87 mg, 96 %). $[\alpha]^{19}_D$ -21.6 (c 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.05 (t, J = 7.8 Hz, 7H), 7.67-7.55 (m, 4H), 7.45 (t, J = 7.5 Hz, 4H), 5.63 (m, 1H), 5.17 (m, 1H), 4.82 (d, J = 11.4 Hz, 1H), 4.45 (d, J = 11.4 Hz, 1H), 4.05 (d, J = 10.3 Hz, 1H), 3.97 (t, J = 10.0 Hz, 1H), 3.71 (d, J = 8.5 Hz, 1H), 3.41 (s, 3H), 2.96 (dd, J = 13.0, 4.8 Hz, 1H), 2.02 (t, J = 12.4 Hz, 1H), 1.61 (d, J = 6.3 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 167.9 (³ $J_{C1,H3ax}$ = 7.2 Hz), 165.2, 165.1, 136.8, 133.6, 133.2, 133.1, 129.7, 129.1, 128.4, 128.3, 128.0, 127.8, 98.7, 72.9, 71.4, 68.6, 66.9, 63.4, 61.02, 52.57, 37.71, 18.5; HRMS (ESI) m/z calcd for: C₃₁H₃₀N₆O₈Na, [M+Na]⁺ 614.2125; found: 614.2132.

Methyl [methyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetra-deoxy-D-glycero- α -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 6)-2,3,4-tri-*O*-benzyl- β -D-galactopyranoside (192eq), and, Methyl [methyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetra-deoxy-D-glycero- β -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 6)-2,3,4-tri-*O*-benzyl- β -D-galactopyranoside (192ax): Glycosylation of acceptor **187** (42 mg, 0.09 mmol, 1.2 eq) with donor **181** (51 mg, 0.075 mmol) was performed according to general procedure **GP** at -78 °C for 6 h to afford after flash column chromatography (cyclohexane/ethyl acetate 85:15) **192eq** (major isomer) and **192ax** (minor isomer) as slightly yellow oils (63 mg, 87% overall, eq:ax 6.7:1, separated after column chromatography). **192eq**: $[\alpha]^{21}_{\text{D}} -3.4$ (c 2.5, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.07-8.03 (m, 4H), 7.62-7.57 (m, 2H), 7.49-7.45 (m, 4H), 7.37-7.32 (m, 7H), 7.31-7.29 (m, 3H), 7.28-7.25 (m, 4H), 7.23-7.20 (m, 1H), 5.52 (dq, $J = 8.2, 6.3$ Hz, 1H), 5.15 (ddd, $J = 12.0, 9.9, 4.9$ Hz, 1H), 4.93 (d, $J = 11.4$ Hz, 1H), 4.88 (d, $J = 11.0$ Hz, 1H), 4.74 (m, 2H), 4.70 (d, $J = 11.8$ Hz, 1H), 4.61 (d, $J = 11.4$ Hz, 1H), 4.29 (d, $J = 7.7$ Hz, 1H), 3.95 (dd, $J = 9.9, 1.5$ Hz, 1H), 3.90 (t, $J = 9.9$ Hz, 1H), 3.87-3.84 (m, 2H), 3.79 (dd, $J = 9.7, 7.7$ Hz, 1H), 3.72 (dd, $J = 8.2, 1.5$ Hz, 1H), 3.59 (dd, $J = 9.2, 8.0$ Hz, 1H), 3.55 (s, 3H), 3.53-3.51 (m, 2H), 3.34 (s, 3H), 2.89 (dd, $J = 12.9, 4.9$ Hz, 1H), 1.91 (dd, $J = 12.9, 12.0$ Hz, 1H), 1.59 (d, $J = 6.3$ Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 167.5 (³ $J_{\text{C1,H3ax}} = 6.4$ Hz), 165.2, 165.0, 138.8, 138.7, 138.5, 133.6, 133.2, 130.0, 129.8, 129.7, 129.1, 128.6, 128.5, 128.3, 128.2, 128.1, 128.1, 127.9, 127.5, 127.4, 127.3, 104.9, 99.0, 82.1, 79.5, 75.1, 74.3, 73.2, 72.9, 72.9, 72.8, 71.5, 68.8, 63.4, 63.0, 60.9, 57.1, 52.7, 37.3, 17.9; HRMS (ESI) m/z calcd for: C₅₂H₅₄N₆O₁₃Na, [M+Na]⁺ 993.3647; found: 993.3619. **192ax**: $[\alpha]^{19}_{\text{D}} -9.6$ (c 0.4, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.05 (d, $J = 7.5$ Hz, 2H), 7.99 (d, $J = 7.5$ Hz, 2H), 7.59 (t, $J = 7.3$ Hz, 1H), 7.53

(t, $J = 7.4$ Hz, 1H), 7.47 (t, $J = 7.6$ Hz, 2H), 7.39-7.26 (m, 16H), 7.18 (t, $J = 7.0$ Hz, 1H), 5.45 (ddd, $J = 11.3, 10.1, 4.9$ Hz, 1H), 5.35 (quintet, $J = 6.2$ Hz, 1H), 4.98 (d, $J = 11.5$ Hz, 1H), 4.87 (d, $J = 11.0$ Hz, 1H), 4.76-4.70 (m, 3H), 4.56 (d, $J = 11.5$ Hz, 1H), 4.17 (d, $J = 7.6$ Hz, 1H), 3.93 (t, $J = 10.1$ Hz, 1H), 3.77-3.74 (m, 2H), 3.73-3.68 (m, 5H), 3.59 (dd, $J = 9.4, 6.5$ Hz, 1H), 3.52 (s, 3H), 3.44-3.38 (m, 3H), 2.76 (dd, $J = 13.0, 4.9$ Hz, 1H), 1.89 (dd, $J = 13.0, 11.3$ Hz, 1H), 1.54 (d, $J = 6.2$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.1 ($^3J_{\text{C1,H3ax}} = 0.0$ Hz), 165.3, 165.3, 138.8, 138.7, 138.5, 133.5, 133.3, 129.7, 129.7, 129.6, 129.2, 128.6, 128.3, 128.2, 128.1, 128.1, 127.8, 127.5, 127.4, 127.3, 104.6, 98.9, 82.2, 79.3, 75.0, 74.6, 74.1, 73.0, 72.5, 71.2, 71.1, 70.8, 63.4, 63.2, 60.9, 56.9, 52.9, 36.9, 17.2; HRMS (ESI) m/z calcd for: $\text{C}_{52}\text{H}_{54}\text{N}_6\text{O}_{13}\text{Na}$, $[\text{M}+\text{Na}]^+$ 993.3647; found: 993.3662.

Methyl [methyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetra-deoxy-*D*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 4)-methyl 7-*O*-acetyl-5-azido-3,5-dideoxy-8,9-*O*-isopropylidene-*D*-glycero-*D*-galacto-non-2-ulopyranoside (193eq), and, Methyl [methyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetra-deoxy-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 4)-methyl 7-*O*-acetyl-5-azido-3,5-dideoxy-8,9-*O*-isopropylidene-*D*-glycero-*D*-galacto-non-2-ulopyranoside (193ax):

Glycosylation of acceptor **186** (53.7 mg, 0.13 mmol, 1.2 eq) with **181** (75 mg, 0.11 mmol) was performed according to general procedure **GP** at -78 °C for 6 h to afford after flash column chromatography over silica gel (hexane/ethyl acetate 4:1) **193eq** (major isomer) and **193ax** (minor isomer) as colorless oils (82.8 mg, 82% overall, eq:ax 3.8:1, separated after column chromatography). **193eq**: $[\alpha]_{\text{D}}^{21}$ 36.4 (c 1.0, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 8.04 (dd, $J = 12.8, 4.8$ Hz, 4H), 7.58 (m, 2H), 7.46 (t, $J = 7.7$ Hz, 4H), 5.55 (dq, $J = 12.8, 6.3$ Hz, 1H), 5.46 (dd, $J = 6.7, 1.4$ Hz, 1H), 5.15 (ddd, $J = 11.8,$

10.0, 4.7 Hz, 1H), 4.49 (ddd, $J = 11.2, 9.5, 5.0$ Hz, 1H), 4.32 (q, $J = 6.2$ Hz, 1H), 4.04 (dd, $J = 8.8, 6.1$ Hz, 1H), 3.96 (t, $J = 9.7$ Hz, 1H), 3.90 (dd, $J = 8.8, 6.1$ Hz, 1H), 3.77 (d, $J = 1.9$ Hz, 1H), 3.76 (s, 3H), 3.70 (d, $J = 6.6$ Hz, 1H), 3.69 (dd, $J = 10.6, 1.5$ Hz, 1H), 3.42 (s, 3H), 3.27 (s, 3H), 3.11 (t, $J = 9.8$ Hz, 1H), 2.91 (dd, $J = 12.6, 4.7$ Hz, 1H), 2.16 (s, 3H), 2.11 (dd, $J = 12.3, 5.1$ Hz, 1H), 1.95 (t, $J = 12.3$ Hz, 1H), 1.64 (dd, $J = 12.4, 4.2$ Hz, 1H), 1.59 (d, $J = 6.3$ Hz, 1H), 1.39 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.0, 167.7, 167.6, 165.2, 164.9, 133.6, 133.1, 130.3, 129.8, 129.6, 129.0, 128.6, 128.4, 109.1, 98.6, 98.2, 74.1, 71.5, 71.4, 70.5, 70.3, 69.8, 66.4, 61.2, 60.8, 52.9, 52.5, 51.1, 38.0, 36.6, 26.6, 25.5, 20.8, 17.8; HRMS (ESI) m/z calcd for: $\text{C}_{40}\text{H}_{47}\text{N}_9\text{O}_{16}\text{Na}$, $[\text{M}+\text{Na}]^+$ 932.3038; found: 932.3030. **193ax**: $[\text{a}]_{\text{D}}^{21}$ 18.6 (c 1.0, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 8.07 (m, 4H), 7.59 (t, $J = 7.3$ Hz, 2H), 7.41 (m, 4H), 5.51 (m, 2H), 5.38 (dd, $J = 7.3, 1.4$ Hz, 1H), 4.33 (dd, $J = 12.8, 6.2$ Hz, 1H), 4.17 (m, 1H), 4.05 (dd, $J = 8.7, 6.0$ Hz, 1H), 3.90 (m, 3H), 3.86 (s, 3H), 3.80 (s, 3H), 3.61 (dd, $J = 10.6, 1.5$ Hz, 1H), 3.30 (d, $J = 6.3$ Hz, 1H), 3.20 (t, $J = 9.8$ Hz, 1H), 3.13 (s, 3H), 2.94 (dd, $J = 13.3, 4.8$ Hz, 1H), 2.56 (dd, $J = 12.5, 5.0$ Hz, 1H), 2.23 (s, 3H), 1.94 (t, $J = 12.6$ Hz, 1H), 1.78 (dd, $J = 12.4, 4.1$ Hz, 1H), 1.68 (d, $J = 6.4$ Hz, 1H), 1.39 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 170.1, 167.0, 166.3 ($^3J_{\text{C1,H3ax}} = 0.0$ Hz), 165.5, 165.2, 133.6, 133.3, 129.9, 129.8, 129.6, 129.1, 128.5, 128.4, 109.2, 100.0, 98.1, 76.7, 73.8, 73.6, 73.5, 72.2, 70.6, 66.7, 61.1, 60.9, 52.9, 52.7, 51.1, 38.8, 37.6, 26.7, 25.5, 20.1, 15.9; HRMS (ESI) m/z calcd for: $\text{C}_{40}\text{H}_{47}\text{N}_9\text{O}_{16}\text{Na}$, $[\text{M}+\text{Na}]^+$ 932.3038; found: 932.3021.

Methyl [methyl (5,7-diazido-3,4,5,7,8,9-hexadeoxy-D-glycero- α -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,4,6-tri-*O*-benzyl- β -D-galactopyranoside (194eq), and, Methyl [methyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetradecoxy-D-glycero- β -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,6-di-*O*-benzyl- β -D-galactopyranoside (194ax): Glycosylation of acceptor **188** (83.6 mg, 0.18 mmol, 1.2 eq) and donor **181** (100 mg, 0.15 mmol) and was performed according to general procedure **GP** at -78 °C for 6 h to afford after flash column chromatography (hexane/ethyl acetate 4:1) an inseparable mixture of isomers (120.7 mg; Yield: 84 %). A solution of this mixture of isomers (120 mg; 0.12 mmol) in methanol (10 mL) was treated with NaOMe (6.67 mg; 0.12 mmol). The resulting mixture was stirred at room temperature until completion. The reaction mixture was neutralized with Amberlyst-15 H⁺ ion exchange resin, filtered and evaporated. The residue was subjected to column chromatography on silica gel (hexane/ethyl acetate 97:3) to obtain compound **194eq** (major isomer) and compound **194ax** (minor isomer) as colorless oils (80.3 mg; 88%, 75% overall for 2 steps, eq:ax 4.5:1, separated after column chromatography). **194eq**: $[\alpha]_D^{21}$ 18.2 (c 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.56-7.17 (m, 15H), 4.86 (d, *J* = 11.2 Hz, 1H), 4.79 (d, *J* = 11.6 Hz, 1H), 4.64 (d, *J* = 11.2 Hz, 1H), 4.49 (dd, *J* = 11.7, 5.4 Hz, 2H), 4.37 (d, *J* = 11.8 Hz, 1H), 4.26 (d, *J* = 7.7 Hz, 1H), 4.05 (m, 1H), 3.95 (dd, *J* = 9.9, 3.0 Hz, 1H), 3.77 (s, 3H), 3.61 (m, 7H), 3.53 (s, 3H, Gal-OMe), 3.45 (t, *J* = 9.8 Hz, 1H), 3.04 (dd, *J* = 9.0, 2.3 Hz, 1H), 2.49 (dd, *J* = 13.7, 4.6 Hz, 1H), 2.05 (dd, *J* = 13.3, 10.9 Hz, 1H), 1.35 (d, *J* = 6.2 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 168.9 (³*J*_{Cl,H3ax} = 7.2 Hz), 138.5, 138.2, 128.3, 128.2, 128.1, 127.9, 127.6, 127.5, 127.4, 127.3, 105.0, 100.4, 77.4, 76.7, 75.9, 74.9, 74.7, 73.3, 72.9, 69.9, 68.9, 65.8, 65.3, 63.4, 63.3, 57.0, 53.6, 37.6, 20.3; HRMS

(ESI) m/z calcd for: $C_{38}H_{46}N_6O_{11}Na$, $[M+Na]^+$ 762.3225; found: 762.3234. **194ax**: $[a]_D^{21}$ 11.6 (c 1.0, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$) δ 7.32 (m, 15H), 4.77 (d, $J = 11.9$ Hz, 1H), 4.72 (d, $J = 11.2$ Hz, 1H), 4.62 (d, $J = 11.2$ Hz, 1H), 4.59 (d, $J = 11.9$ Hz, 1H), 4.43 (d, $J = 11.2$ Hz, 1H), 4.38 (d, $J = 11.2$ Hz, 1H), 4.22 (d, $J = 7.6$ Hz, 1H), 4.17 (d, $J = 2.2$ Hz, 1H), 4.08 (d, $J = 7.6$ Hz, 1H), 4.17 (d, $J = 2.2$ Hz, 1H), 4.08 (d, $J = 10.4$ Hz, 1H), 4.05 (d, $J = 2.5$ Hz, 1H), 4.03 (d, $J = 2.1$ Hz, 1H), 3.67 (m, 3H), 3.53 (m, 2H), 3.49 (s, 3H), 3.38 (t, $J = 10.0$ Hz, 1H), 3.35 (s, 3H), 3.01 (d, $J = 9.1$ Hz, 1H), 2.60 (dd, $J = 13.8, 4.8$ Hz, 1H), 1.78 (dd, $J = 13.7, 11.7$ Hz, 1H), 1.13 (d, $J = 6.1$ Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.8 ($^3J_{C1,H3ax} = 0.0$ Hz), 138.8, 138.5, 137.2, 128.8, 128.6, 128.2, 127.5, 127.2, 105.5, 99.2, 76.8, 76.2, 75.2, 74.6, 73.7, 72.9, 71.4, 68.6, 68.1, 64.6, 63.8, 57.0, 52.3, 39.9, 21.8; HRMS (ESI) m/z calcd for: $C_{38}H_{46}N_6O_{11}Na$, $[M+Na]^+$ 762.3225; found: 762.3116.

Methyl [methyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetra-deoxy-D-glycero- α -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,6-di-*O*-benzyl- β -D-galactopyranoside (195eq), and, Methyl [methyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetra-deoxy-D-glycero- β -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,6-di-*O*-benzyl- β -D-galactopyranoside (195ax): Glycosylation of acceptor **189** (67 mg, 0.18 mmol, 1.2 eq) with **181** (101 mg, 0.15 mmol) was performed according to general procedure **GP** at -78 °C for 6 h to afford after flash column chromatography (cyclohexane/ethyl acetate 4:1) **195eq** (major isomer) and a mixture separable by HPLC (gradient elution hexane/ethyl acetate from 95:5 to 7:3) of **195ax** (minor isomer) and **196** (eq-glycoside (2 \rightarrow 4)) as slightly yellow oils (116 mg, 88 %, eq:ax:(eq_(4-OH)) 4.7:1:(0.9), separated after column chromatography and HPLC). **195eq**: $[a]_D^{22}$ 15.3 (c 4.45, $CHCl_3$); 1H NMR (600 MHz,

CDCl₃) δ 8.02-7.99 (m, 4H), 7.62-7.60 (m, 1H), 7.58-7.55 (m, 1H), 7.48-7.43 (m, 4H), 7.37-7.32 (m, 4H), 7.27-7.24 (m, 3H), 7.06-7.03 (m, 2H), 6.97-6.95 (m, 1H), 5.42 (dq, $J = 8.6, 6.3$ Hz, 1H), 5.27 (ddd, $J = 11.8, 9.0, 5.0$ Hz, 1H), 4.80 (d, $J = 10.6$ Hz, 1H), 4.64 (d, $J = 11.9$ Hz, 1H), 4.61 (d, $J = 11.9$ Hz, 1H), 4.51 (d, $J = 10.6$ Hz, 1H), 4.33 (d, $J = 7.8$ Hz, 1H), 4.05 (dd, $J = 9.6, 3.3$ Hz, 1H), 3.89 (s, 1H), 3.86-3.82 (m, 3H), 3.79 (dd, $J = 10.2, 6.3$ Hz, 1H), 3.72 (dd, $J = 8.6, 1.6$ Hz, 1H), 3.68 (t, $J = 5.7$ Hz, 1H, *Gal-H-5*), 3.58 (s, 3H), 3.53 (dd, $J = 9.6, 7.8$ Hz, 1H), 3.35 (s, 3H), 2.74 (dd, $J = 13.3, 5.0$ Hz, 1H), 2.61 (s, 1H), 2.05 (dd, $J = 13.3, 11.8$ Hz, 1H), 1.49 (d, $J = 6.3$ Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 167.8 (³ $J_{\text{C1,H3ax}} = 7.0$ Hz), 165.0, 164.8, 138.3, 138.1, 133.5, 133.3, 129.9, 129.8, 129.7, 129.1, 128.5, 128.5, 128.4, 128.2, 128.1, 127.6, 127.6, 127.5, 104.7, 99.2, 77.5, 75.3, 75.3, 73.6, 72.9, 72.7, 71.6, 69.6, 69.3, 68.4, 63.8, 60.7, 56.9, 53.0, 36.1, 18.1; HRMS (ESI) m/z calcd for: C₄₅H₄₈N₆O₁₃Na, [M+Na]⁺ 903.3177; found: 903.3167.

195ax: [a]_D²² 10.9 (c 0.65, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.05-8.04 (m, 4H), 7.60-7.55 (m, 2H), 7.47-7.43 (m, 4H), 7.38 (d, $J = 7.5$ Hz), 7.30-7.27 (m, 6H), 7.25-7.20 (m, 2H), 5.62 (ddd, $J = 11.4, 10.0, 4.5$ Hz, 1H), 5.54-5.50 (m, 1H), 4.75 (d, $J = 11.0$ Hz, 1H), 4.66 (d, $J = 11.0$ Hz, 1H), 4.58-4.53 (m, 2H), 4.36 (dd, $J = 10.0, 1.2$ Hz, 1H), 4.24 (d, $J = 7.6$ Hz, 1H), 3.97-3.95 (m, 1H), 3.91-3.88 (m, 2H), 3.73 (dd, $J = 9.5, 3.1$ Hz, 1H), 3.70 (dd, $J = 10.2, 5.4$ Hz, 1H), 3.62 (dd, $J = 10.2, 5.4$ Hz, 1H), 3.57 (s, 3H), 3.52-3.50 (m, 4H), 3.45 (t, $J = 5.4$ Hz, 1H), 2.95 (dd, $J = 13.2, 4.5$ Hz, 1H), 2.80 (d, $J = 5.1$ Hz, 1H), 1.97 (dd, $J = 13.2, 11.4$ Hz, 1H), 1.54 (d, $J = 6.3$ Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 166.9 (³ $J_{\text{C1,H3ax}} = 0.0$ Hz), 165.4, 165.3, 138.6, 137.9, 133.5, 133.2, 129.9, 129.8, 129.7, 129.3, 128.5, 128.5, 128.3, 128.2, 128.1, 127.7, 127.6, 127.4, 104.7, 99.7,

78.5, 77.3, 75.1, 73.6, 72.8, 72.0, 71.6, 71.1, 69.4, 68.6, 64.0, 61.1, 57.0, 52.8, 36.8, 16.6; HRMS (ESI) m/z calcd for: $C_{45}H_{48}N_6O_{13}Na$, $[M+Na]^+$ 903.3177; found: 903.3151.

Methyl [methyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetradecoxy-*D*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 4)-2,6-di-*O*-benzyl- β -*D*-galactopyranoside

(196): This compound was isolated as a minor byproduct from the glycosylation of **189** with **181** as described above. $[\alpha]_D^{21}$ 22.2 (c 0.7, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$) δ 8.05 (d, $J = 7.7$ Hz, 2H), 8.00 (d, $J = 7.7$ Hz, 2H), 7.62-7.56 (m, 2H), 7.49-7.44 (m, 4H), 7.40 (d, $J = 7.5$ Hz, 2H), 7.33-7.30 (m, 6H), 7.27-7.23 (m, 2H), 5.45-5.41 (m), 5.14 (ddd, $J = 11.9, 10.0, 4.7$ Hz, 1H), 4.86 (d, $J = 11.3$ Hz, 1H), 4.77 (d, $J = 11.3$ Hz, 1H), 4.57 (d, $J = 12.2$ Hz, 1H), 4.52 (d, $J = 12.2$ Hz, 1H), 4.33 (d, $J = 2.5$ Hz, 1H), 4.25 (d, $J = 7.6$ Hz, 1H), 3.99 (d, $J = 8.2$ Hz, 1H), 3.90 (t, $J = 10.0$ Hz, 1H), 3.63 (d, $J = 10.5$ Hz, 1H), 3.61-3.58 (m, 2H), 3.52 (s, 3H), 3.51-3.45 (m, 2H), 3.40 (dd, $J = 9.6, 7.6$ Hz, 1H), 3.26 (s, 3H), 3.21 (d, $J = 5.9$ Hz, 1H), 3.05 (dd, $J = 13.0, 4.7$ Hz, 1H), 2.01 (dd, $J = 13.0, 11.9$ Hz, 1H), 1.58 (d, $J = 6.2$ Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.9 ($^3J_{C1,H3ax} = 6.3$ Hz), 165.2, 164.9, 138.8, 138.2, 133.6, 133.2, 130.0, 129.8, 129.6, 129.0, 128.6, 128.5, 128.4, 128.2, 128.1, 127.6, 127.5, 127.4, 104.7, 98.8, 79.8, 74.7, 73.5, 73.4, 73.1, 72.4, 72.2, 71.4, 69.8, 69.1, 64.4, 60.7, 57.1, 52.8, 37.8, 17.7; HRMS (ESI) m/z calcd for: $C_{45}H_{48}N_6O_{13}Na$, $[M+Na]^+$ 903.3177; found: 903.3174.

Methyl [1-adamantanyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetradecoxy-*D*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate] (197eq), and, Methyl [1-adamantanyl (5,7-diazido-4,8-di-*O*-benzoyl-3,5,7,9-tetradecoxy-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate] (197ax): Glycosylation of 1-adamantanol **190** (14 mg, 0.090 mmol, 1.2 eq) with **181** (51 mg, 0.075 mmol) was performed according to general procedure **GP**

at -78 °C for 9 h to afford after flash column chromatography (hexane/acetone 95:5) compound **197eq** (major isomer) and compound **197ax** (minor isomer) as slightly yellow oils (36 mg, 73% overall, eq:ax 4.2:1, separated after column chromatography). **197eq**: $[\alpha]_D^{19}$ -5.4 (c 1.3, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.04-8.02 (m, 4H), 7.59-7.56 (m, 2H), 7.47-7.44 (m, 4H), 5.51 (dq, *J* = 9.7, 6.2 Hz, 1H), 5.00 (ddd, *J* = 12.3, 10.2, 4.5 Hz, 1H), 4.29 (dd, *J* = 10.2, 1.7 Hz, 1H), 3.90 (t, *J* = 10.2 Hz, 1H), 3.63 (dd, *J* = 9.6, 1.7 Hz, 1H), 3.41 (s, 3H), 2.82 (dd, *J* = 12.7, 4.5 Hz, 1H), 2.10 (br s, 3H), 1.94 (t, *J* = 12.7 Hz, 1H), 1.79 (d, *J* = 2.4 Hz, 6H), 1.60 (d, *J* = 6.2 Hz, 3H), 1.59 (br s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 170.3 (³*J*_{C1,H3ax} = 5.7 Hz), 165.2, 165.1, 133.5, 132.9, 130.3, 129.7, 129.6, 129.2, 128.5, 128.3, 97.9, 79.0, 72.7, 71.1, 68.3, 63.9, 61.2, 52.2, 43.2, 40.8, 36.0, 31.0, 18.5; HRMS (ESI) *m/z* calcd for: C₃₄H₃₈N₆O₈Na, [M+Na]⁺ 681.2649; found: 681.2632. **197ax**: $[\alpha]_D^{22}$ -10.8 (c 0.4, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.05-8.03, 7.59-7.56 (m, 2H), 7.47-7.44 (m, 4H), 5.64 (ddd, *J* = 11.2, 10.1, 4.7 Hz, 1H), 5.42 (dq, *J* = 7.6, 6.2 Hz, 1H), 4.13 (dd, *J* = 10.1, 1.1 Hz, 1H), 3.88 (t, *J* = 10.1 Hz, 1H), 3.74 (s, 3H), 3.71 (dd, *J* = 7.6, 1.1 Hz, 1H), 2.78 (dd, *J* = 12.6, 4.7 Hz, 1H), 1.87 (br s, 3H), 1.72-1.67 (m, 7H), 1.65 (d, *J* = 6.2 Hz, 3H), 1.41 (d, *J* = 12.3 Hz, 3H), 1.29 (d, *J* = 11.8 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 169.5 (³*J*_{C1,H3ax} = 0.0 Hz), 165.4, 165.2, 133.4, 133.4, 129.7, 129.7, 129.4, 128.5, 128.5, 96.9, 78.2, 71.1, 71.0, 70.1, 63.7, 61.4, 52.4, 42.6, 40.3, 35.7, 30.7, 17.6; HRMS (ESI) *m/z* calcd for: C₃₄H₃₈N₆O₈Na, [M+Na]⁺ 681.2649; found: 681.2624.

Methyl [methyl (5,7-diazido-3,4,5,7,8,9-hexadeoxy-D-glycero-α-D-galacto-non-2-ulopyranosid)onate]-(2→6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (198): To a solution of **192eq** (130 mg; 0.13 mmol) in methanol (15 mL) was added NaOMe (7.23

mg; 0.13 mmol). The resulting mixture was stirred at room temperature until complete conversion. The reaction mixture was neutralized with Amberlyst-15 H⁺ resin and filtered. The filtrate was evaporated and subjected to column chromatography over silica gel (Hexane/Ethyl acetate 6:4) to give **198** as colorless oil (89 mg; 87%). [α]_D²¹ 20.9 (c 1.0, CHCl₃); ¹H NMR: δ 7.28 (m, 15H), 4.97 (d, *J* = 11.6 Hz, 1H), 4.87 (d, *J* = 10.9 Hz, 1H), 4.75 (m, 2H), 4.71 (d, *J* = 11.3 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.25 (d, *J* = 7.7 Hz, 1H), 4.12 (m, 1H), 3.83 (d, *J* = 2.7 Hz, 1H), 3.80 (m, 1H), 3.77 (t, *J* = 9.9 Hz, 1H), 3.61 (s, 3H), 3.59 (dd, *J* = 10.2, 5.4 Hz, 1H), 3.54 (s, 3H), 3.48 (m, 5H), 3.13 (dd, *J* = 9.1, 2.1 Hz, 1H), 2.68 (dd, *J* = 13.2, 4.7 Hz, 1H), 1.85 (dd, *J* = 12.8, 10.6 Hz), 1.40 (d, *J* = 6.2 Hz, 1H); ¹³C NMR: δ 168.8, 138.7, 138.4, 128.3, 128.2, 128.1, 128.1, 127.6, 127.6, 127.5, 127.4, 127.3, 104.9, 98.8, 81.9, 79.5, 75.1, 74.2, 73.2, 73.1, 72.8, 72.6, 69.6, 65.8, 65.8, 63.8, 62.6, 57.1, 53.4, 39.4, 20.3; *m/z* calcd for: C₃₈H₄₆N₆O₁₁Na, [M+Na]⁺ 762.3225; found: 762.3241.

Methyl [methyl (5,7-diazido-3,4,5,7,8,9-hexadeoxy-D-glycero-α-D-galacto-non-2-uloypyranosid)onate]-(2→3)-2,6-di-O-benzyl-β-D-galactopyranoside (203): To a solution of **195eq** (50 mg; 0.06 mmol) in methanol (10 mL) was added NaOMe (3 mg; 0.06). The resulting mixture was stirred at room temperature until complete conversion. Then the reaction mixture was quenched by Amberlyst-15 H⁺ resin and filtered. The filtrate was evaporated and subjected to column chromatography over silica gel (Hexane/Ethyl acetate 6:4) to give **203** as a colorless oil (27.1 mg; 72%). [α]_D²¹ 30.4 (c 1.0, CHCl₃); ¹H NMR: δ 7.32 (m, 10H), 4.85 (d, *J* = 11.2 Hz, 1H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.58 (m, 2H), 4.28 (d, *J* = 7.7 Hz, 1H), 4.06 (dd, *J* = 8.7, 6.2 Hz, 1H), 3.94 (dd, *J* = 9.6, 3.1 Hz, 1H), 3.81 (s, 3H), 3.75 (m, 4H), 3.59 (m, 5H), 3.44 (t, *J* = 10.1 Hz, H-5).

3.08 (dd, $J = 8.9, 2.0$ Hz, 1H), 2.52 (dd, $J = 13.6, 4.6$ Hz, 1H), 2.05 (t, $J = 11.6$ Hz, 1H), 1.35 (d, $J = 6.2$ Hz, 3H); ^{13}C NMR: δ 169.0, 138.4, 128.4, 128.2, 127.7, 127.6, 127.5, 104.8, 99.8, 77.3, 77.0, 76.7, 75.5, 74.9, 73.6, 73.1, 72.5, 69.8, 69.6, 65.4, 63.4, 57.0, 53.7, 38.0, 20.3; m/z calcd for: $\text{C}_{31}\text{H}_{40}\text{N}_6\text{O}_{11}\text{Na}$, $[\text{M}+\text{Na}]^+$ 695.2653; found: 695.2661.

Methyl [methyl (4,8-di-*O*-acetyl-5,7-diacetamido-3,5,7,9-tetradecoxy-D-glycero- α -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 6)-2,3,4-tri-*O*-benzyl- β -D-galactopyranoside

(199): To a solution of **198** (70 mg; 0.09 mmol) in 1:1 dioxane:water (8 mL) was added 10% Pd/C (70 mg) followed by glacial acetic acid (0.25 mL). The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h then filtered and was evaporated to dryness. Pyridine (5 mL) and acetic anhydride (5 mL) were added to the residue and the resulting mixture was stirred at room temperature for 10 h. The solvents were evaporated and the residue was subjected to column chromatography over silica gel (dichloromethane/methanol 94:6) to give **199** (45 mg; 68%). $[\alpha]_{\text{D}}^{21}$ 26.2 (c 1.0, CHCl_3); ^1H NMR: δ 6.53 (d, $J = 10.0$ Hz, 1H), 5.71 (d, $J = 7.9$ Hz, 1H), 5.61 (d, $J = 7.8$ Hz, 1H), 5.36 (m, 1H), 5.20 (dd, $J = 10.6, 7.9$ Hz, 1H), 4.96 (dd, $J = 10.6, 6.2$ Hz, 1H), 4.94 (dd, $J = 8.7$ Hz, 3.5 Hz, 1H), 4.63 (d, $J = 8.0$ Hz, 1H), 4.30 (t, $J = 9.8$ Hz, 1H), 4.10 (dd, $J = 9.3, 6.9$ Hz, 1H), 3.84 (s, 3H), 3.60 (dd, $J = 12.1, 9.4$ Hz, 1H), 3.51 (s, 3H), 3.31 (m, 1H), 2.63 (dd, $J = 12.6, 4.7$ Hz, 1H), 2.15 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H), 1.67 (t, $J = 12.4$ Hz, 1H), 1.28 (d, $J = 6.2$ Hz, 3H); ^{13}C NMR: δ 171.9, 171.4, 170.7, 170.5, 170.4, 170.2, 169.6, 166.6, 101.7, 100.7, 72.3, 71.0, 70.0, 68.5, 67.3, 67.2, 66.8, 64.2, 57.1, 53.0, 52.0, 51.9, 38.7, 29.7, 23.6, 22.9, 21.4, 20.9, 20.8, 20.7, 17.7; m/z calcd for: $\text{C}_{31}\text{H}_{46}\text{N}_2\text{O}_{18}\text{Na}$, $[\text{M}+\text{Na}]^+$ 757.2643; found: 757.2641.

Methyl [methyl (4,8-di-*O*-acetyl-5,7-diacetamido-3,5,7,9-tetra-deoxy-D-glycero- α -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,4,6-tri-*O*-benzyl- β -D-galactopyranoside

(201): Method 1 (From 194eq): To a solution of **194eq** (40 mg; 0.05 mmol) in 1:1 dioxane:water (6 mL) was added 10% Pd/C (70 mg) followed by glacial acetic acid (0.12 mL). The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h, then filtered off, and evaporated to dryness. Pyridine (5 mL) and acetic anhydride (5 mL) were added to the residue and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated and the residue was subjected to column chromatography over silica gel (dichloromethane/methanol 94:6) to give **201** (28.1 mg; 73%). Method 2 (From 203): To a solution of **203** (20 mg; 0.03 mmol) in 1:1 dioxane:water (3 mL) was added 10% Pd/C (20 mg) followed by glacial acetic acid (0.06 mL). The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h, then filtered off, and evaporated to dryness. Pyridine (3 mL) and acetic anhydride (3 mL) were added to the residue and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated and the residue was subjected to column chromatography over silica gel (dichloromethane/methanol 94:6) to give **201** (14.3 mg; 66%). $[\alpha]_D^{21}$ 37.5 (c 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 6.35 (s, 1H), 5.34 (d, J = 8.9 Hz, 1H), 5.28 (s, 1H), 5.21 (m, 1H), 5.10 (dd, J = 12.3, 6.1 Hz, 1H), 5.03 (d, J = 2.9 Hz, 1H), 4.64 (d, J = 8.3 Hz, 1H), 4.55 (dd, J = 10.0, 3.2 Hz, 1H), 4.51 (d, J = 7.6 Hz, 1H), 4.33 (t, J = 10.2 Hz, 1H), 4.08 (d, J = 6.3 Hz, 1H), 3.83 (s, 3H), 3.52 (s, 3H), 2.64 (dd, J = 12.4, 4.6 Hz, 1H), 2.17 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.90 (s, 3H), 1.56 (t, J = 12.2 Hz, 1H), 1.24 (d, J = 6.2 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.8, 170.8, 170.3, 170.3, 170.3, 170.21, 170.19, 167.55,

101.48, 96.79, 71.03, 71.03, 70.82, 68.36, 68.36, 67.89, 67.46, 62.05, 56.97, 53.10, 51.37, 51.05, 37.51, 23.40, 23.02, 21.73, 20.84, 20.82, 20.66, 20.66, 17.79; m/z calcd for: $C_{31}H_{46}N_2O_{18}Na$, $[M+Na]^+$ 757.2643; found: 757.2662.

Methyl [5,7-Diacetamido-3,5,7,9-tetra-deoxy-D-glycero- α -D-galacto-non-2-ulopyranosid)onic acid]-(2 \rightarrow 6)- β -D-galactopyranoside (200): To a solution of **199** (30 mg; 0.04 mmol) in H_2O (3.0 mL) was added saturated aq $Ba(OH)_2$ (1.0 mL). The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and saturated with CO_2 . The precipitate was filtered off and the filtrate was frozen using a dry ice-acetone bath and lyophilized to obtain the white foam **200** (18.1 mg, 91 %). $[a]_D^{21}$ 3.3 (c 0.5, H_2O); 1H NMR (600 MHz, D_2O) δ 4.15 (d, $J = 8.0$ Hz, 1H), 3.82 (m, 2H), 3.76 (m, 3H), 3.68 (dd, $J = 9.3, 2.9$ Hz, 1H), 3.61 (dd, $J = 8.1, 4.2$ Hz, 1H), 3.49 (m, 2H), 3.43 (m, 4H) 3.33 (dd, $J = 9.9, 8.0$ Hz, 1H), 2.58 (dd, $J = 12.4, 4.5$ Hz, 1H), 1.83 (s, 3H), 1.78 (s, 3H), 1.52 (t, $J = 12.1$ Hz, 1H), 1.00 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 173.9, 173.6, 173.3, 103.8, 100.4, 73.4, 72.5, 71.6, 70.6, 68.7, 68.6, 67.1, 63.7, 57.3, 53.9, 52.1, 40.2, 23.2, 21.9, 18.0; m/z calcd for: $C_{20}H_{33}N_2O_{13}$, $[M-H]^-$ 509.1983; found: 509.1962.

Methyl [5,7-Diacetamido-3,5,7,9-tetra-deoxy-D-glycero- α -D-galacto-non-2-ulopyranosid)onic acid]-(2 \rightarrow 3)- β -D-galactopyranoside (202): To a solution of **201** (15 mg; 0.02 mmol) in H_2O (1.5 mL) was added saturated aq $Ba(OH)_2$ (1.0 mL). The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and saturated with CO_2 . The precipitate was filtered off and the filtrate was frozen using a dry ice-acetone bath and lyophilized to obtain the white foam **202** (9.2 mg, 92 %). $[a]_D^{21}$ 1.8 (c 0.5, H_2O); 1H NMR (600 MHz, D_2O) δ 4.19 (d, $J = 7.9$ Hz, 1H),

3.91 (m, 1H), 3.79 (d, $J = 6.4$ Hz, 1H), 3.75 (s, 1H), 3.65 (m, 2H), 3.56 (m, 3H), 3.48 (t, $J = 9.8$ Hz, 1H), 3.40 (s, 3H), 3.37 (d, $J = 8.0$ Hz, 1H), 2.58 (dd, $J = 12.8, 4.3$ Hz, 1H), 1.81 (s, 3H), 1.77 (s, 3H), 0.99 (d, $J = 6.2$ Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 173.9, 173.8, 173.7, 103.5, 99.5, 75.8, 74.8, 71.7, 69.0, 68.6, 67.2, 67.0, 60.9, 57.0, 53.8, 51.9, 40.0, 22.4, 21.9, 18.0; m/z calcd for: $\text{C}_{20}\text{H}_{33}\text{N}_2\text{O}_{13}$, $[\text{M}-\text{H}]^-$ 509.1983; found: 509.1996.

Methyl (1-adamantyl 4,7,8,9-tetra-*O*-acetyl-5-*O*-levulinoyl-3-deoxy-2-thio-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate (214), and, Methyl (1-adamantyl 7,8,9-tri-*O*-acetyl-4,5-di-*O*-levulinoyl-3-deoxy-2-thio-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate (215): A solution of sialoside **160**¹³¹ (1 g ; 1.56 mmol) in dry dichloromethane (14 mL) was treated with dry pyridine (1.14 mL; 14.04 mmol) under argon and cooled to -10 °C. After stirring for 15 min, crushed nitrosyl tetrafluoroborate (0.65 g; 2.62 mmol) was added in one portion. The reaction mixture was stirred for -10 °C until TLC/MS showed complete conversion, then was diluted with cold dichloromethane and washed with cold 1 N HCl, saturated aq. NaHCO_3 and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under 10 °C to obtain the nitrosated sialoside **212**, which was carried forward without any further purification. A solution of the crude nitrosated sialoside **212** (1.06 g; 1.59 mmol) in dry dichloromethane (16 mL) and 2,2,2-trifluoroethanol (184 μL ; 2.38 mmol) under argon at -10 °C was stirred for 0.5 h and then treated with freshly prepared sodium isopropoxide in isopropanol (0.2 N; 9.49 mL; 1.91 mmol). The resulting mixture was stirred for 2 min, then treated with a cold solution of levulinic acid (3.23 mL; 31.77 mmol) in 32 mL of dry dichloromethane. After stirring for 5 min, the reaction mixture was warmed to 0 °C and quenched with saturated aq. NaHCO_3 . It was then diluted with dichloromethane,

washed with cold brine, dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 10% acetone in toluene to afford **214** (535 mg; 49%) and **215** (243 mg; 21%). **Compound 214**: [α]²¹_D -36.9 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.41 (t, *J* = 2.3 Hz, 1H), 5.36 (ddd, *J* = 11.9, 9.6, 4.9 Hz, 1H), 5.21 (dt, *J* = 8.7, 1.6 Hz, 1H), 4.96 (dd, *J* = 12.4, 1.6 Hz, 1H), 4.87 (t, *J* = 9.8 Hz, 1H), 4.72 (dd, *J* = 10.0, 2.6 Hz, 1H), 4.25 (dd, *J* = 12.4, 8.6 Hz, 1H), 3.82 (s, 3H), 2.82 - 2.69 (m, 1H), 2.65 - 2.52 (m, 3H), 2.49 - 2.37 (m, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.02-1.96 (m, 9H), 1.92 - 1.84 (m, 4H), 1.71 - 1.62 (m, 8H); ¹³C NMR (101 MHz, CDCl₃) δ 205.9, 171.7, 170.7, 170.4, 170.2, 170.1, 169.8, 86.0, 73.2, 71.2, 69.0, 68.6, 68.2, 63.2, 63.2, 52.8, 50.5, 43.8, 43.4, 39.6, 37.7, 36.1, 35.9, 29.9, 29.8, 29.6, 27.9, 21.0, 20.8, 20.6; ESI-HRMS (C₃₃H₄₆NaO₁₄S): [M+Na]⁺ *m/z*: 721.2506; found: 721.2486. **Compound 215**: [α]²¹_D -28.1 (c 0.51, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.40 (t, *J* = 2.2, 1H), 5.36 (ddd, *J* = 11.9, 9.7, 4.9 Hz, 1H), 5.20 (dt, *J* = 8.8, 1.6 Hz, 1H), 4.96 (dd, *J* = 12.3, 1.5 Hz, 1H), 4.87 (t, *J* = 9.8 Hz, 1H), 4.71 (dd, *J* = 10.0, 2.6 Hz, 1H), 4.25 (dd, *J* = 12.4, 8.7 Hz, 1H), 3.81 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.02 - 1.96 (m, 9H), 1.92 - 1.80 (m, 4H), 1.65 (br s, 8H); ¹³C NMR (101 MHz, CDCl₃) δ 206.6, 206.2, 171.7, 170.8, 170.4, 170.2, 169.8, 86.0, 73.2, 71.2, 69.0, 68.9, 68.1, 63.2, 52.8, 50.5, 43.4, 39.5, 37.7, 35.9, 29.8, 29.7, 27.9, 27.8, 21.0, 20.6; ESI-HRMS (C₃₆H₅₀NaO₁₅S): [M+Na]⁺ *m/z*: 777.2768; found: 777.2766.

Methyl (1-adamantyl 4,7,8,9-tetra-*O*-acetyl-3-deoxy-2-thio-*D*-glycero- β -*D*-galactonon-2-ylpyranosid)onate (216): To a solution of **214** (0.56 gm; 0.80 mmol) in 30 mL of dry dichloromethane at room temperature under argon was added 1.9 mL of dry

pyridine. This was followed by addition of hydrazine monohydrate (0.11 mL; 3.18 mmol) and glacial acetic acid (1.42 mL). The resulting mixture was stirred at room temperature for 1 h. Then the reaction mixture was quenched with 10 mL of acetone and further stirred for 15 min. The reaction mixture was then diluted with dichloromethane and washed with water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 45% EtOAc in hexane to afford **216** (0.36 g; 77%). $[\alpha]_{\text{D}}^{21}$ -54.8 (c 0.81, CHCl_3): ^1H NMR (400 MHz, CDCl_3) δ 5.53 (dd, $J = 3.7, 2.6$ Hz, 1H), 5.36 - 5.22 (m, 2H), 4.84 (dd, $J = 12.5, 1.6$ Hz, 1H), 4.43 (dd, $J = 9.6, 2.5$ Hz, 1H), 4.33 (dd, $J = 12.5, 7.1$ Hz, 1H), 3.80 (s, 3H), 3.22 (t, $J = 9.5$ Hz, 1H), 3.10 (br s, 1H), 2.60 (dd, $J = 13.6, 4.8$ Hz, 1H), 2.18 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.98 (d, $J = 11.2$ Hz, 6H), 1.88 - 1.81 (m, 4H), 1.79 (dd, $J = 13.4, 11.9$ Hz, 1H), 1.65 (s, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.5, 170.7, 170.5, 170.3, 170.1, 85.8, 73.0, 72.1, 71.0, 70.7, 69.2, 62.7, 52.7, 50.3, 43.4, 39.4, 35.9, 29.8, 21.1, 21.0, 20.8, 20.7; ESI-HRMS ($\text{C}_{28}\text{H}_{40}\text{NaO}_{12}\text{S}$): $[\text{M}+\text{Na}]^+$ m/z 623.2138; found 623.2137; ESI-HRMS ($\text{C}_{28}\text{H}_{40}\text{NaO}_{12}\text{S}$): $[\text{M}+\text{Na}]^+$ m/z : 623.2138; found: 623.2137.

Methyl (1-adamantyl 7,8,9-tri-*O*-acetyl-3-deoxy-2-thio-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate (217): To a solution of **215** (150 mg; 0.21 mmol) in 20 mL of dry dichloromethane at room temperature under argon was added 0.7 mL of dry pyridine. This was followed by addition of hydrazine monohydrate (28 μL ; 0.81 mmol) and glacial acetic acid (0.5 mL). The resulting mixture was stirred at room temperature for 1 h. Then the reaction mixture was quenched with 10 mL of acetone and further stirred for 15 min. The reaction mixture was then diluted with dichloromethane and washed with

water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with EtOAc in hexane to afford the pale yellow oil **217** (87 mg; 78%). $[\alpha]_{\text{D}}^{21}$ -55 (c 0.82, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 5.42 (dd, $J = 3.7, 2.2$ Hz, 1H), 5.30 (ddd, $J = 6.6, 3.8, 1.5$ Hz, 1H), 4.88 (dd, $J = 12.6, 1.4$ Hz, 1H), 4.36 (dd, $J = 12.4, 6.9$ Hz, 1H), 4.33 (dd, $J = 9.5, 2.2$ Hz, 1H), 4.11 (ddd, $J = 12.0, 8.9, 4.7$ Hz, 1H), 3.80 (s, 3H), 3.39 (br s, 1H), 2.96 (t, $J = 9.3$ Hz, 1H), 2.78 (br s, 1H), 2.54 (dd, $J = 13.8, 4.7$ Hz, 1H), 2.19 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.96 (d, $J = 12.5$ Hz, 7H), 1.81 (d, $J = 10.7$ Hz, 4H), 1.75 (dd, $J = 13.7, 12.1$ Hz, 2H), 1.64 (s, 7H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.4, 170.5, 170.3, 170.3, 86.1, 72.7, 72.1, 71.9, 71.5, 67.8, 62.7, 52.7, 50.1, 43.4, 41.3, 35.9, 29.7, 21.1, 20.9, 20.7; ESI-HRMS ($\text{C}_{26}\text{H}_{38}\text{NaO}_{11}\text{S}$): $[\text{M}+\text{Na}]^+$ m/z : 581.2033; found: 581.2020.

Methyl (1-adamantyl 4,7,8,9-tetra-*O*-acetyl-5-*O*-triflyl-3-deoxy-2-thio- β -D-galacto-non-2-ulopyranosid)onate (223) : Method 1: A solution of sialoside **160 (50 mg; 0.08 mmol) in dry dichloromethane (1 mL) was treated with dry pyridine (63 μL ; 0.78 mmol) under argon and cooled to -10 °C. After stirring for 15 min, crushed nitrosyl tetrafluoroborate (36 mg; 2.62 mmol) was added in one portion. The reaction was stirred for -10 °C until TLC/MS showed complete conversion, then was diluted with cold dichloromethane and washed with cold 1 N HCl, saturated aq. NaHCO_3 and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under 10 °C to obtain the nitrosated sialoside **212** which was carried forward without any further purification. 18-crown-6 (47 mg; 0.18 mmol) and sodium 2,2,2-trifluoroethoxide (2 mg; 0.164 mmol) were dissolved in anhydrous dichloromethane (0.4 mL) under argon,**

cooled to -10 °C and added to the crude nitrosyl sialoside **212** (55 mg; 0.082 mmol) in anhydrous dichloromethane (0.8 mL) at -10 °C under argon. After 2 min, triflic acid (0.37 ml; 4.1 mmol) was added to the reaction mixture. The mixture was stirred for 5 minutes and quenched with methanol (2 mL). The volatiles were evaporated and the crude compound was purified by column chromatography on silica gel (eluent: 25% EtOAc in hexane) to afford **223** (19 mg, 34%). **Method 2:** To a solution of **216** (100 mg; 0.156 mmol) in 5 mL of dry dichloromethane was added dry pyridine (50 μ L; 0.62 mmol) and the temperature was lowered to -78 °C. At -78 °C, Tf₂O (52 μ L; 0.312 mmol) was added and the mixture was stirred for 15 min. Then the mixture was brought to 0 °C and further stirred for 2 h, before it was diluted with dichloromethane, washed with 1 N HCl and water. Then the organic layer was dried (Na₂SO₄) and concentrated to give a yellowish solid, which was then subjected to column chromatography (25% EtOAc in hexane) to give **223** (98 mg; 81%). $[\alpha]_D^{21}$ -72.1 (c 1.0, CHCl₃) ¹H NMR (400 MHz, CDCl₃) δ 5.45 (ddd, J = 11.7, 9.1, 5.1 Hz, 1H), 5.36 (dt, J = 8.8, 4.4 Hz, 1H), 5.32 - 5.25 (m, 1H), 5.01 (t, J = 9.4 Hz, 1H), 4.76 (dd, J = 9.6, 2.3 Hz, 1H), 4.68 (dd, J = 12.3, 2.1 Hz, 1H), 4.24 (dd, J = 12.4, 7.1 Hz, 1H), 3.85-3.76 (m, 2H), 2.72 (dd, J = 13.7, 5.1 Hz, 1H), 2.14 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.91 - 1.82 (m, 4H), 1.66 (s, 6H), 1.43(m, 5H); ¹³C NMR (101 MHz, CDCl₃) δ 170.8, 170.5, 169.4, 169.3, 169.2, 85.3, 79.9, 71.7, 70.0, 69.0, 68.2, 62.8, 52.9, 50.9, 43.5, 43.3, 39.4, 35.9, 29.8, 29.7, 20.6, 20.5; ESI-HRMS (C₂₉H₃₉F₃NaO₁₄S₂): [M+Na]⁺ m/z: 755.1601; found: 755.1592

Methyl (1-adamantyl 4,7,8,9-tetra-O-acetyl-5-azido-3,5-dideoxy-2-thio-D-glycero- β -D-gulo-non-2-ulopyranosid)onate (206): To a solution of **223** (201 mg, 0.28 mmol) in 15 mL of dry DMF was added LiN₃ (202 mg; 4.13 mmol). The resulting mixture was

stirred at 0 °C for 24 h, then was diluted with ethyl acetate, and washed with NaHCO₃, water and brine. The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 30% EtOAc in hexane system to afford **206** (127 mg; 74%). $[\alpha]_D^{21}$ -106 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.64 (dd, *J* = 8.8, 3.3 Hz, 1H), 5.38 (ddd, *J* = 11.4, 5.4, 3.3 Hz, 1H), 5.27 (dt, *J* = 6.5, 3.8 Hz, 1H), 4.51 (dd, *J* = 8.9, 1.0 Hz, 1H), 4.43 (dd, *J* = 12.1, 4.2 Hz, 1H), 4.19 (dd, *J* = 12.1, 6.7 Hz, 1H), 4.10 (d, *J* = 1.5 Hz, 1H), 3.78 (s, 3H), 2.35-2.21 (m, 2H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 1.97 (d, *J* = 11.4 Hz, 8H), 1.82 (d, *J* = 11.1 Hz, 3H), 1.65 (s, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 170.6, 169.9, 169.9, 169.8, 169.3, 86.0, 70.7, 69.4, 69.0, 61.6, 58.5, 52.7, 50.4, 43.3, 36.0, 34.2, 29.8, 20.8, 20.7, 20.6, 20.6 ESI-HRMS (C₂₈H₃₉N₃NaO₁₁S): [M+Na]⁺ *m/z*: 648.2203; found: 648.2199.

Methyl (1-adamantyl 4,7,8,9-tetra-*O*-acetyl-5-*O*-levulinic-3-deoxy-2-thio-*D*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate (224): The nitrosyl sialoside **221** (157 mg, 0.23 mmol) was deaminated using the general procedure with 2,2,2-trifluoroethanol (27 μ L, 0.35 mmol), 0.2 N sodium isopropoxide in isopropanol (1.4 mL, 0.28 mmol) and levulinic acid (477 μ L, 4.68 mmol) to afford **224** after flash chromatography over silica gel eluting with 10% acetone in toluene as a colorless oil (67 mg, 41%). $[\alpha]_D^{21}$ +24.5 (c = 0.96, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.28 (s, 2H), 4.87 - 4.78 (m, 2H), 4.30 (d, *J* = 12.3 Hz, 1H), 4.13 (t, *J* = 10.6 Hz, 2H), 3.81 (s, 3H), 2.87-2.77 (m, 1H), 2.76 - 2.70 (m, 1H), 2.60 (dd, *J* = 9.3, 4.0 Hz, 1H), 2.57 - 2.52 (m, 1H), 2.50 - 2.42 (m, 1H), 2.19 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.96 (d, *J* = 12.0 Hz, 4H), 1.86 (d, *J* = 11.7 Hz, 3H), 1.66 (s, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 206.1, 171.7, 170.7,

170.4, 169.9, 169.6, 84.5, 72.8, 69.3, 68.5, 67.8, 66.4, 61.5, 52.8, 51.1, 43.5, 39.6, 37.7, 36.0, 29.9, 29.7, 27.9, 21.1, 20.8, 20.7; ESI-HRMS (C₃₃H₄₆NaO₁₄S): [M+Na]⁺ m/z: 721.2506; found: 721.2491.

Methyl (1-adamantyl 5-azido-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (287): To a solution of **286**^{118,131} (810 mg; 1.73 mmol) in 1:1 MeOH/H₂O (18 mL) was added imidazole-1-sulfonyl azide hydrochloride¹⁶⁵ (0.72 g; 3.46 mmol), K₂CO₃ (717 mg; 5.19 mmol) and CuSO₄·5H₂O (43 mg; 0.17 mmol). The reaction mixture was stirred at room temperature for 3 h. Thereafter, the solvent was evaporated and the residue was purified by flash chromatography on silica gel eluting with 8% MeOH in CHCl₃ to afford the white sticky solid **287** (724 mg; 92%). [α]_D²¹ -48.1 (c 1.0, CHCl₃) ¹H NMR (400 MHz, CD₃OD) δ 4.84 (s, 3H), 4.22 (d, *J* = 10.4 Hz, 1H), 4.06 (ddt, *J* = 11.8, 9.8, 5.9 Hz, 1H), 3.90-3.75 (s, 4H), 3.75 - 3.63 (s, 3H), 3.35 (dd, *J* = 23.4, 13.5 Hz, 1H), 2.33 (dd, *J* = 13.6, 4.7 Hz, 1H), 2.08 - 1.88 (br s, 8H), 1.81 - 1.60 (m, 7H), 1.22(m, 1H); ¹³C NMR (101MHz, CD₃OD); δ 172.4, 85.9, 78.6, 70.9, 70.00, 69.8, 67.9, 63.9, 63.8, 59.8, 52.1, 49.5, 43.0, 35.7, 29.9, 19.9, 13.1; ESI-HRMS (C₂₀H₃₁N₃NaO₇S): [M+Na]⁺ m/z: 480.1796; found: 480.1789.

Methyl (1-adamantyl 4,7,8,9-tetra-O-acetyl-5-azido-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (288) : Method 1: To a solution of **287** (724 mg; 1.58 mmol) in 15 mL of dry pyridine was added acetic anhydride (1.2 mL; 12.66 mmol). The resulting mixture was stirred under argon at room temperature for 4 h, was diluted with ethyl acetate and washed successively with saturated aq. NaHCO₃ solution, 1 N HCl and again with saturated aq. NaHCO₃ solution. The organic layer was then dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by column

chromatography on silica gel eluting with 30% EtOAc in hexane to afford **288** (0.84 g; 85%). **Method 2:** A solution of sialoside **160** (300 mg; 0.468 mmol) in dry dichloromethane (4.6 ml) was treated with dry pyridine (0.38 mL; 4.68 mmol) under argon and cooled to -10 °C. After stirring for 15 min, crushed nitrosyl tetrafluoroborate (218 mg; 1.872 mmol) was added in one portion. The reaction was stirred for -10 °C until TLC/MS showed complete conversion, then was diluted with cold dichloromethane (25 mL) and washed with cold 1 N HCl, saturated aq. NaHCO₃ and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under 10 °C to obtain the nitrosated sialoside **212** which was carried forward without any further purification. A solution of the crude nitrosated sialoside **212** (320 mg; 0.48 mmol) in dry dichloromethane (4.8 mL) and 2,2,2-trifluoroethanol (55 µL; 0.72 mmol) under argon at -10 °C was stirred for 30 min and treated with freshly prepared sodium isopropoxide in isopropanol (0.2 N; 9.49 mL; 1.91 mmol). After stirring for 3 mins, the reaction mixture was treated with 1.7 N Hydrazoic acid in chloroform²³¹ (5.61 mL; 9.54 mmol). The reaction mixture was further stirred at -10 °C for 10 min, warmed to 0 °C and quenched with saturated aq. NaHCO₃. It was then diluted with dichloromethane, washed with cold brine, dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 30% EtOAc in hexane to afford **288** (148 mg; 51%). $[\alpha]_D^{21} -106$ (c 1.0, CHCl₃) ¹H NMR (400 MHz, CDCl₃): δ 5.59 (dd, $J = 3.6, 2.2$ Hz, 1H), 5.32 (ddd, $J = 11.7, 9.8, 4.8$ Hz, 1H), 5.25-5.17 (m, 1H), 4.82 (dd, $J = 12.5, 1.6$ Hz, 1H), 4.29 (ddd, $J = 15.8, 10.6, 4.7$ Hz, 2H), 3.79 (s, 3H), 3.19 (t, $J = 10.0$ Hz, 1H), 2.67 (dd, $J = 13.5, 4.8$ Hz, 1H), 2.18 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.03 (s, 1H), 2.02 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.85 - 1.72 (m, 4H), 1.62 (s, 6H), 1.25 (t, J

= 7.1 Hz, 2H); ^{13}C NMR (101 MHz, CDCl_3): δ 170.5, 170.4, 169.8, 169.7, 169.4, 85.7, 72.3, 71.0, 70.5, 70.1, 62.6, 60.4, 52.8, 50.6, 43.3, 39.3, 35.9, 29.7, 21.1, 20.9, 20.7; ESI-HRMS ($\text{C}_{28}\text{H}_{39}\text{N}_3\text{NaO}_{11}\text{S}$): $[\text{M}+\text{Na}]^+$ m/z : 648.2203; found: 648.2187

General Coupling Protocol with Donors 206 or 288: A mixture of donor **206** or **288** (0.15 mmol), acceptor (0.18 mmol) and activated 4Å acid-washed powdered molecular sieves (300 mg; 2 g/mmol of donor) in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (2:1, 2 mL) was stirred for 2 h at room temperature, then was cooled to $-78\text{ }^\circ\text{C}$ and was treated with NIS (42 mg; 0.18 mmol) and TfOH (2 μL ; 0.02 mmol). The reaction mixture was stirred at $-78\text{ }^\circ\text{C}$ for 5 h and then quenched with triethylamine (7 μL). The mixture was diluted with CH_2Cl_2 , filtered through Celite, washed with 20% aqueous $\text{Na}_2\text{S}_2\text{O}_3$, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with mixture of EtOAc and hexane to afford the desired coupled products.

Methyl (cyclohexyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-azido-*D*-glycero- α -*D*-gulonon-2-ulopyranosid)onate (231): Compound **231** was prepared according to the general glycosylation procedure using donor **206** (25 mg; 0.04 mmol) and compound **229** as acceptor (5 μL ; 0.05 mmol) in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (1.0 mL; 2:1) at $-78\text{ }^\circ\text{C}$. After chromatographic purification (gradient elution of EtOAc/Hexanes 2% to 30%) **231** (17.6 mg; 79%) was obtained as white foam. $[\alpha]_{\text{D}}^{21}$ -11.6 (c 1.0, CHCl_3); ^1H NMR (600 MHz, CDCl_3): δ 5.53 - 5.40 (dd, $J = 6.0, 4.4$ Hz, 1H), 5.26 (td, $J = 5.8, 3.0$ Hz, 1H), 4.92 - 4.85 (m, 1H), 4.36 (dd, $J = 12.4, 2.9$ Hz, 1H), 4.21 (dd, $J = 12.4, 5.5$ Hz, 1H), 3.96 (dd, $J = 4.3, 0.9$ Hz, 1H), 3.91 (d, $J = 2.5$ Hz, 1H), 3.75 (t, $J = 3.5$ Hz, 1H), 3.70-3.61 (m, 1H), 2.38 (dd, $J = 12.8, 4.5$ Hz, 1H), 2.20 (dd, $J = 12.1, 6.5$, 1H), 2.11 (s, 3H), 2.10 (s, 3H),

2.05 (s, 3H), 1.87 (t, $J = 7.3$ Hz, 2H), 1.74 - 1.64 (m, 2H), 1.60 (d, $J = 11.8$ Hz, 2H), 1.47 (dd, $J = 8.8, 4.0$ Hz, 2H), 1.41 - 1.02 (m, 6H); ^{13}C NMR (151 MHz, CDCl_3): δ 170.6, 170.1, 170.0, 169.9, 169.0 ($^3J_{\text{C-H}} = 6.9$), 99.0, 74.2, 70.3, 70.1, 70.0, 69.1, 61.6, 58.9, 52.6, 34.5, 33.2, 32.8, 25.4, 24.4, 24.3, 21.0, 20.8, 20.7, 20.6; ESI-HRMS ($\text{C}_{24}\text{H}_{35}\text{N}_3\text{NaO}_{12}$): $[\text{M}+\text{Na}]^+$ m/z : 580.2118; found: 580.2109.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-azido-*D*-glycero- α -*D*-gulonon-2-ulopyranosid)onate]-(2 \rightarrow 6)-2,3,4-tri-*O*-benzyl- β -*D*-galactopyranoside (232):

Compound **232** was prepared according to the general glycosylation procedure using donor **206** (45 mg; 0.07 mmol) and acceptor **187** (40.07 mg; 0.08 mmol) in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (2.0 mL; 2:1) at -78 °C. After chromatographic purification (gradient elution of EtOAc / Hexanes 2% to 30%) compound **232** (47.5 mg; 72%) was obtained as a white foam. $[\alpha]_{\text{D}}^{21} -18.4$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.37 - 7.30 (m, 15H), 5.43 (dd, $J = 6.3, 4.6$ Hz, 1H), 5.26 (dd, $J = 5.6, 3.0$ Hz, 1H), 4.99 - 4.90 (m, 2H), 4.86 (d, $J = 11.0$ Hz, 1H), 4.78 - 4.67 (m, 3H), 4.63 (t, $J = 7.8$ Hz, 1H), 4.34 (d, $J = 3.0$ Hz, 1H), 4.32 (d, $J = 3.0$ Hz, 1H), 4.28 (d, $J = 7.7$ Hz, 1H), 4.20 - 4.14 (m, 1H), 3.95 (dd, $J = 7.1$ Hz, 3.7 Hz, 2H), 3.89 (d, $J = 2.7$ Hz, 1H), 3.80 (dt, $J = 7.1, 3.7$ Hz, 1H), 3.79 - 3.74 (m, 2H), 3.64 - 3.59 (m, 3H), 3.53 (d, $J = 4.9$ Hz, 3H), 2.38 (dd, $J = 12.8, 4.6$ Hz, 1H), 2.21 (t, $J = 12.8$ Hz, 1H), 2.16 (t, $J = 4.5$ Hz, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.6, 170.0, 169.9, 169.8, 168.0 ($^3J_{\text{C-H}} = 6.6$), 138.9, 138.8, 138.5, 128.3, 128.2, 128.1, 128.0, 127.6, 127.4, 127.4, 127.2, 105.0, 98.8, 82.5, 79.6, 75.1, 74.3, 73.2, 72.9, 72.6, 70.5, 70.3, 69.9, 68.9, 62.3, 61.5, 58.8, 57.0, 52.8, 32.2, 29.7, 20.9, 20.8, 20.7, 20.6; ESI-HRMS ($\text{C}_{46}\text{H}_{55}\text{N}_3\text{NaO}_{17}$): $[\text{M}+\text{Na}]^+$ m/z : 944.3429; found: 944.3409.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-azido-*D*-glycero- α -*D*-gulo-non-2-ulopyranosid)onate]-(2→3)-2,4,6-tri-*O*-benzyl- β -*D*-galactopyranoside (233):

Compound **233** was prepared according to the general glycosylation procedure using donor **206** (40 mg; 0.06 mmol) and acceptor **188** (35.63 mg; 0.07 mmol) in CH₂Cl₂/CH₃CN (1.8 mL; 2:1) at -78 °C. After chromatographic purification (gradient elution of EtOAc / Hexanes 2% to 30%) compound **233** (33.6 mg; 57%) was obtained as a white foam. $[\alpha]_D^{21}$ -11.6 (c 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.43 - 7.15 (m, 15H), 5.39 (dd, *J* = 6.9, 3.4 Hz, 1H), 5.29 - 5.22 (m, 1H), 5.07 - 5.01 (m, 1H), 4.95 (d, *J* = 11.6 Hz, 1H), 4.83 (d, *J* = 11.1 Hz, 1H), 4.65 (d, *J* = 11.1 Hz, 1H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.41 (d, *J* = 11.6 Hz, 1H), 4.36 - 4.30 (m, 2H), 4.04 (dd, *J* = 12.4, 5.1 Hz, 1H), 3.98 (dd, *J* = 10.0, 2.9 Hz, 1H), 3.92 (s, 1H), 3.87 (d, *J* = 1.9 Hz, 1H), 3.83 - 3.80 (m, 1H), 3.78 (d, *J* = 2.8 Hz, 1H), 3.69 (s, 3H), 3.63 (m, 2H), 3.55 (s, 3H), 2.46 (t, *J* = 13.0 Hz, 1H), 2.21 (dd, *J* = 13.5, 4.9 Hz, 1H), 2.16 (d, *J* = 5.9 Hz, 1H), 2.08 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.88 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.5, 169.9, 169.8, 169.6, 168.2 (³*J*_{C-H} = 7.1), 139.3, 138.5, 138.1, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 127.1, 104.9, 100.0, 77.5, 76.2, 75.1, 74.7, 73.5, 73.4, 70.2, 69.9, 69.7, 69.0, 68.9, 61.3, 59.3, 57.0, 52.8, 29.6, 20.9, 20.7, 20.6; ESI-HRMS (C₄₆H₅₅N₃NaO₁₇): [M+Na]⁺ m/z: 944.3429; found: 944.3412.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-azido-*D*-glycero- α -*D*-gulo-non-2-ulopyranosid)onate]-(2→4)-2,3,6-tri-*O*-benzyl- β -*D*-galactopyranoside (234):

Compound **234** was prepared according to the general glycosylation procedure using donor **206** (30 mg; 0.04 mmol) and acceptor **230** (30 mg; 0.05 mmol) in CH₂Cl₂/CH₃CN (1.2 mL; 2:1) at -78 °C. After chromatographic purification (gradient elution of

EtOAc/Hexanes 2% to 30%) compound **234** (19 mg, 44%) was obtained as a colorless oil. $[\alpha]_D^{21}$ -10.2 (c 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.53 - 7.06 (m, 15H), 5.54 (t, *J* = 5.6 Hz, 1H), 5.40 - 5.30 (m, 2H), 5.10 (s, 1H), 4.77 (d, *J* = 10.9 Hz, 1H), 5.40 - 5.30 (m, 2H), 4.73 - 4.66 (m, 1H), 4.60 (d, *J* = 11.0 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.42 - 4.36 (m, 1H), 4.31 - 4.25 (m, 1H), 4.22 (d, *J* = 7.3 Hz, 1H), 4.12 (s, 1H), 4.06 (dd, *J* = 12.3, 6.4 Hz, 1H), 3.98 (s, 3H), 3.92 - 3.80 (m, 2H), 3.68 (s, 3H), 3.58 (dd, *J* = 18.0, 10.9 Hz, 1H), 3.50 (s, 2H), 2.42-2.30 (m, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.6, 170.1, 169.8, 169.8, 167.7 (³*J*_{C-H} = 7.2), 138.6, 137.8, 137.6, 128.4, 128.2, 128.1, 128.0, 127.6, 127.5, 104.9, 98.7, 78.3, 75.1, 73.5, 73.4, 72.6, 71.1, 69.4, 69.3, 68.9, 68.1, 67.3, 61.6, 60.0, 58.9, 57.1, 52.1, 21.0, 20.9, 20.7, 20.6; ESI-HRMS (C₄₆H₅₅N₃NaO₁₇): [M+Na]⁺ *m/z*: 944.3429; found: 944.3408.

Methyl (cyclohexyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-azido-*D*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate (289): Method 1: Compound **289 was prepared according to the general glycosylation procedure using donor **288** (40 mg; 0.06 mmol) and acceptor **229** (8 μ L; 0.07 mmol) in CH₂Cl₂/CH₃CN (1.8 mL; 2:1) at -78 °C. After chromatographic purification (gradient elution of EtOAc / Hexanes 2% to 30%) compound **289** (28.4 mg; 81%) was obtained as a white foam. Method 2: Compound **289** was also prepared according to the general glycosylation procedure using donor **296** (75 mg; 0.12 mmol) and acceptor **229** (15 μ L; 0.15 mmol) in CH₂Cl₂/CH₃CN (3.0 mL; 2:1) at -78 °C. After chromatographic purification (gradient elution of EtOAc/Hexanes 2% to 30%) **289** (57.2 mg; 86%) was obtained as a white foam. $[\alpha]_D^{21}$ -9.2; (c 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 5.49 (d, *J* = 12.6 Hz, 1H), 4.21 (dd, *J* = 12.5, 4.4**

Hz, 1H), 3.78 (t, $J = 5.1$ Hz, 3H), 3.69 - 3.60 (m, 1H), 3.19 (t, $J = 10.2$ Hz, 1H), 2.71 (dd, $J = 12.7, 4.6$ Hz, 1H), 2.17 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.88 (s, 1H), 1.71 (dd, $J = 13.1$ Hz, 8.7 Hz, 2H), 1.66 (d, $J = 14.3$ Hz, 2H), 1.55 (d, $J = 10.1$ Hz, 2H), 1.48 (d, $J = 12.3$ Hz, 2H), 1.33 (dd, $J = 10.3, 6.3$ Hz, 2H), 1.26 - 1.18 (m, 2H), 1.11 (d, $J = 9.2$ Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.7, 169.8, 169.7, 168.4 ($^3J_{\text{C-H}} = 6.2$), 98.4, 74.3, 71.4, 71.1, 68.1, 67.9, 61.9, 60.1, 52.5, 38.0, 34.7, 33.0, 25.4, 24.2, 24.1, 21.0, 20.9, 20.8, 20.7; ESI-HRMS ($\text{C}_{24}\text{H}_{35}\text{N}_3\text{NaO}_{12}$): $[\text{M}+\text{Na}]^+$ m/z : 580.2118; found: 580.2109.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-azido-*D*-glycero- α -*D*-galactonon-2-ylpyranosid)onate]-(2 \rightarrow 6)-2,3,4-tri-*O*-benzyl- β -*D*-galactopyranoside (204):

Compound **204** was prepared according to the general glycosylation procedure using donor **288** (45 mg; 0.07 mmol) and acceptor **187** (40.1 mg; 0.09 mmol) in CH_2Cl_2 / CH_3CN (2.0 mL; 2:1) at -78 °C. After chromatographic purification (gradient elution of EtOAc/Hexanes 2% to 30%) compound **204** (48.8 mg; 74%) was obtained as a white foam. $[\alpha]_{\text{D}}^{21} -15.7$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.43 - 7.14 (m, 15H), 5.48 (d, $J = 9.1$ Hz, 1H), 5.34 (dd, $J = 9.0, 3.2$ Hz, 1H), 4.96 - 4.92 (m, 1H), 4.87 (d, $J = 10.9$ Hz, 1H), 4.81 (dd, $J = 12.0, 4.9$ Hz, 1H), 4.76 - 4.68 (m, 2H), 4.65 (t, $J = 9.9$ Hz, 1H), 4.30 (d, $J = 2.1$ Hz, 1H), 4.27 (dd, $J = 5.1, 2.6$ Hz, 1H), 4.18 (d, $J = 4.3$ Hz, 1H), 4.16 (d, $J = 4.4$ Hz, 1H), 3.88 (dd, $J = 8.8, 5.7$ Hz, 1H), 3.84 (d, $J = 3.2$ Hz, 1H), 3.80 - 3.75 (m, 2H), 3.61 (s, 3H), 3.55 (s, 3H), 3.50 (m, 2H), 3.28-3.18 (m, 1H), 2.70 (dd, $J = 12.9$ Hz, 4.8 Hz, 1H), 2.23 (d, $J = 4.4$ Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.75 (t, $J = 12.4$ Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.7, 169.7, 169.7, 169.6, 167.4 ($^3J_{\text{C-H}} = 6.4$), 138.8, 138.7, 138.5, 128.3, 128.2, 128.1, 128.0, 127.6,

127.5, 127.4, 127.2, 104.9, 99.0, 82.0, 79.5, 75.1, 74.2, 73.3, 72.9, 72.6, 71.6, 71.1, 68.0, 67.8, 52.9, 61.8, 59.9, 57.0, 52.8, 37.3, 21.0, 20.9, 20.8, 20.7; ESI-HRMS ($C_{46}H_{55}N_3NaO_{17}$): $[M+Na]^+$ m/z: 944.3429; found: 944.3401.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-azido-*D*-glycero- α -*D*-galactono-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,4,6-tri-*O*-benzyl- β -*D*-galactopyranoside (290):

Compound **290** was prepared according to the general glycosylation procedure using donor **288** (35 mg; 0.05 mmol) and acceptor **188** (31.2 mg; 0.06 mmol) in CH_2Cl_2/CH_3CN (1.5 mL; 2:1) at $-78\text{ }^\circ\text{C}$. After chromatographic purification (gradient elution of EtOAc / Hexanes 2% to 30%) compound **290** (33.9 mg; 66%) was obtained as a white foam. $[\alpha]_D^{21} -17.8$ (c 1.0, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$) δ 7.50 - 6.99 (m, 15H), 5.45 (dd, $J = 9.2, 1.1$ Hz, 1H), 5.40 (ddd, $J = 9.3, 3.9, 2.2$ Hz, 1H), 4.90 - 4.84 (m, 2H), 4.80 (d, $J = 11.2$ Hz, 1H), 4.66 (d, $J = 11.3$ Hz, 1H), 4.51 (d, $J = 11.8$ Hz, 1H), 4.44 (d, $J = 11.5$ Hz, 1H), 4.38 (d, $J = 11.6$ Hz, 1H), 4.30 (dd, $J = 4.9, 2.7$ Hz, 1H), 4.28 (d, $J = 2.1$ Hz, 1H), 4.02 (d, $J = 4.0$ Hz, 1H), 4.00 (d, $J = 4.0$ Hz, 1H), 3.96 (d, $J = 2.9$ Hz, 1H), 3.94 (d, $J = 3.0$ Hz, 1H), 3.70 (s, 3H), 3.64 - 3.59 (m, 4H), 3.54 (s, 3H), 3.51 (d, $J = 3.9$ Hz, 1H), 3.15 (t, $J = 10.2$ Hz, 1H), 2.61 (dd, $J = 13.3, 4.8$ Hz, 1H), 2.11 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 169.5, 169.4, 167.6 ($^3J_{C-H} = 6.8$), 139.1, 138.8, 138.0, 128.3, 128.1, 128.0, 127.8, 127.7, 127.7, 127.4, 127.1, 104.9, 98.8, 77.5, 76.3, 76.2, 74.9, 74.8, 73.5, 73.1, 71.4, 71.2, 68.5, 68.2, 67.7, 61.6, 59.8, 57.1, 52.9, 41.7, 35.9, 35.4, 29.4, 28.5, 21.0, 20.8, 20.7, 20.4; ESI-HRMS ($C_{46}H_{55}N_3NaO_{17}$): $[M+Na]^+$ m/z: 944.3429; found: 944.3403.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-azido-*D*-glycero- α -*D*-galactono-2-ulopyranosid)onate]-(2 \rightarrow 4)-2,3,6-tri-*O*-benzyl- β -*D*-galactopyranoside (291):

Compound **291** was prepared according to the general glycosylation procedure using donor **288** (40 mg; 0.0639 mmol) and acceptor **230** (35.63 mg; 0.0767 mmol) in CH₂Cl₂/CH₃CN (1.8 mL; 2:1) at -78 °C. After chromatographic purification on silica gel (gradient elution of EtOAc/Hexanes 2% to 30%) compound **291** (23.2 mg; 41%) was obtained as a colorless oil. $[\alpha]_D^{21}$ -21.4 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.45 - 7.14 (m, 15H), 5.57 (s, 2H), 5.50 - 5.35 (m, 1H), 4.86 - 4.72 (m, 3H), 4.59 (d, *J* = 10.9 Hz, 1H), 4.47 (dd, *J* = 18.1, 12.2 Hz, 3H), 4.34 - 4.24 (m, 1H), 4.19 (d, *J* = 7.7 Hz, 1H), 4.13 - 4.02 (m, 2H), 3.89 - 3.81 (m, 1H), 3.81 - 3.72 (m, 1H), 3.62 - 3.54 (m, 1H), 3.49 (s, 3H), 3.44 (s, 3H), 3.31 (dd, *J* = 16.2, 10.9 Hz, 2H), 2.65 (dd, *J* = 12.9, 4.9 Hz, 1H), 2.23 (s, 3H), 2.12 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.6, 170.4, 170.1, 169.4, 167.7 (³*J*_{C-H} = 6.9), 138.1, 137.3, 128.6, 128.6, 128.4, 128.2, 128.0, 127.9, 127.4, 127.4, 105.2, 98.4, 82.0, 78.5, 75.1, 73.4, 73.3, 72.5, 70.7, 69.6, 69.1, 68.3, 66.8, 62.0, 60.9, 57.1, 51.9, 38.7, 21.2, 20.9; ESI-HRMS (C₄₆H₅₅N₃NaO₁₇): $[M+Na]^+$ *m/z*: 944.3429; found: 944.3407.

Methyl (1-adamantyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-*N*-(1,1-dimethylethoxy)carbonyl-2-thio-*D*-glycero- α -*D*-galactono-2-ulopyranosid)onate (292):

To a solution of **220** (1.5 g; 2.35 mmol) in anhydrous THF (10 mL) were added di-*tert*-butyl dicarbonate (5.11 g, 23.52 mmol) and DMAP (114 mg; 0.95 mmol) at room temperature. The mixture was stirred for 10 h at 60 °C under argon before it was cooled to room temperature and concentrated under reduced pressure. The residue was applied to silica gel and eluted with 35% EtOAc in hexanes to give **292** (1.3 g; 76%). $[\alpha]_D^{21}$

+42.3 (c 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 5.32 - 5.22 (m, 2H), 5.19 - 5.10 (m, 1H), 4.86 (t, *J* = 10.5 Hz, 1H), 4.69 (d, *J* = 10.3 Hz, 1H), 4.32 (m, 1H), 4.04 (dd, *J* = 12.6, 3.7 Hz, 1H), 3.77 (s, 3H), 2.76 (m, 1H), 2.35 (s, 3H), 2.17 (s, 3H), 2.05 (s, 3H), 1.99 (m, 6H), 1.93 (s, 3H), 1.88 (s, 3H), 1.65 (m, 6H), 1.54 (s, 9H), 1.49 (m, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 173.9, 170.6, 170.1, 170.1, 169.9, 169.6, 151.8, 84.7, 84.3, 73.0, 69.0, 67.2, 66.5, 61.4, 52.6, 52.4, 51.2, 43.5, 41.2, 36.0, 27.9, 27.8, 26.7, 21.2, 20.1, 20.7, 20.6; ESI-HRMS (C₃₅H₅₁NNaO₁₄S): [M+Na]⁺ *m/z*: 764.2928; found: 764.2936.

Methyl (1-adamantyl 5-azido-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-non-2-uloxyranosid)onate (295): To a solution of **292** (1.25 g; 1.68 mmol) in dry methanol (7 mL) was added a catalytic amount of sodium methoxide. The solution was stirred for 1 h at room temperature and then quenched with Amberlyst 15 ion-exchange resin. The mixture was filtered through Celite and concentrated under reduced pressure to give a crude preparation of **293** (0.9 g), which was dissolved in 8 mL of dry THF and was treated with 2 N HCl in diethyl ether (6 mL) at 0 °C. The resulting mixture was brought to room temperature and stirred for 5 h, and then the mixture was concentrated under reduced pressure to give crude a crude preparation of **294** (0.75 g, 1.6 mmol), which was dissolved in 1:1 MeOH:H₂O (16 mL) followed by addition of imidazole-1-sulfonyl azide hydrochloride (0.66 g; 3.2 mmol), K₂CO₃ (0.66 g; 4.8 mmol) and CuSO₄·5H₂O (40 mg; 0.16 mmol). The reaction mixture was stirred at room temperature for 3 h. Thereafter, the solvent was evaporated and the residue was purified by flash chromatography on silica gel eluting with 8% MeOH in CHCl₃ to afford the white sticky solid **295** (0.66 g; 86% for 3 steps). [α]²¹_D +20.6 (c 1.0, MeOH); ¹H NMR (400 MHz, CD₃OD) δ 3.84 (s,

3H), 3.65 (m, 3H), 3.56 - 3.40 (m, 3H), 3.31 (m, 1H), 2.64 (dd, $J = 8.3, 4.2$ Hz, 1H), 2.08 (m, 9H), 1.72 (m, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 171.9, 84.6, 75.1, 71.8, 69.5, 68.9, 63.3, 62.8, 59.1, 52.2, 50.1, 43.4, 42.1, 41.7, 39.5, 35.7, 31.5, 29.9; ESI-HRMS ($\text{C}_{20}\text{H}_{31}\text{N}_3\text{NaO}_7\text{S}$): $[\text{M}+\text{Na}]^+$ m/z : 480.1796; found: 480.1775.

Methyl (1-adamantyl 4,7,8,9-tetra-*O*-acetyl-5-azido-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-non-2-ulopyranosid)onate (296): **Method 1:** To a solution of **295** (420 mg; 1.30 mmol) in 8 mL of dry pyridine was added acetic anhydride (0.99 mL; 10.44 mmol). The resulting mixture was stirred under argon at room temperature for 4 h, then was diluted with ethyl acetate and washed successively with saturated aq NaHCO_3 solution, 1 N HCl and again with saturated aq NaHCO_3 solution. The organic layer was then dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 30% EtOAc in hexane to afford **296** (480 mg; 84%). **Method 2:** A solution of sialoside **220** (300 mg; 0.47 mmol) in dry dichloromethane (4.6 mL) was treated with dry pyridine (0.38 mL; 4.68 mmol) under argon and cooled to -10 °C. After stirring for 15 min, crushed nitrosyl tetrafluoroborate (218 mg; 1.87 mmol) was added in one portion. The reaction was stirred for -10 °C until TLC/MS showed complete conversion, then was diluted with cold dichloromethane (25 mL) and washed with cold 1 N HCl, saturated NaHCO_3 and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under 10 °C to give the nitrosated sialoside **221** which was carried forward without any further purification. A solution of the crude nitrosated sialoside **221** (320 mg; 0.48 mmol) in dry dichloromethane (4.8 mL) and 2,2,2-trifluoroethanol (55 μL ; 0.72 mmol) under argon at -10 °C was stirred for 30 min and treated with freshly prepared sodium isopropoxide in isopropanol (0.2 N; 9.49

mL; 1.91 mmol). After stirring for 3 mins, the reaction mixture was treated with 1.7 N hydrazoic acid in chloroform²³¹ (5.61 mL; 9.54 mmol). The reaction mixture was further stirred at -10 °C for 10 min, warmed to 0 °C and quenched with saturated aq NaHCO₃. It was then diluted with dichloromethane, washed with cold brine, dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 30% EtOAc in hexane to afford **296** (135 mg; 46%). [α]_D²¹ +63.2 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.48 (d, *J* = 9.8 Hz, 1H), 5.28 (d, *J* = 9.8 Hz, 1H), 4.69 (t, *J* = 9.5 Hz, 1H), 4.25 (dd, *J* = 11.4, 4.6 Hz, 2H), 3.79 (s, 3H), 3.16 (t, *J* = 10.1 Hz, 1H), 2.75 (dd, *J* = 12.7, 4.6 Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.86 (m, 9H), 1.65 (m, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 170.7, 170.0, 169.9, 169.6, 169.6, 84.1, 72.8, 71.2, 68.4, 67.6, 61.5, 60.1, 52.8, 51.2, 43.5, 39.3, 35.9, 29.9, 21.0, 20.9, 20.8; ESI-HRMS (C₂₈H₃₉N₃NaO₁₁S): [M+Na]⁺ *m/z*: 648.2203; found: 648.2180.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-acetamido-*D*-glycero- α -*D*-gulo-non-2-ulopyranosid)onate]-(2 \rightarrow 6)-2,3,4-tri-*O*-acetyl- β -*D*-galactopyranoside (297): To a solution of **232** (50 mg, 0.06 mmol) in 10 mL of 1,4-dioxane/water (1:1) was added 50 mg of 5% Pd/C (100 wt%) followed by 0.18 mL of glacial acetic acid. The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h. Then the solution was filtered off and the solvents were evaporated. To the residue, 5 mL of Ac₂O and 5 mL of pyridine were added and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated and the residue was subjected to column chromatography on silica gel (5% MeOH in CH₂Cl₂) to afford the colorless oil **297** (35 mg; 82%). [α]_D²¹ -13.8 (c 0.5, CHCl₃); ¹H NMR (600 MHz, CDCl₃)

δ 6.16 (d, $J = 9.6$ Hz, 1H), 5.61 (d, $J = 3.3$ Hz, 1H), 5.46 (dd, $J = 7.0, 3.8$ Hz, 1H), 5.17 (dd, $J = 10.5, 7.9$ Hz, 1H), 5.11 - 5.03 (m, 2H), 4.81 - 4.73 (m, 1H), 4.69 - 4.62 (m, 1H), 4.41 (t, $J = 9.4$ Hz, 1H), 4.21 (dt, $J = 12.7, 6.4$ Hz, 1H), 4.16 (dd, $J = 11.8, 6.1$ Hz, 1H), 3.97 (dd, $J = 7.0, 1.2$ Hz, 1H), 3.91 - 3.86 (m, 1H), 3.79 (s, 3H), 3.78 - 3.74 (m, 1H), 3.57 - 3.50 (m, 1H), 3.48 (s, 3H), 2.35 (dd, $J = 13.4, 4.7$ Hz, 1H), 2.14 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.1, 170.8, 170.4, 170.2, 170.1, 170.0, 169.6, 169.5, 167.5, 102.0, 99.3, 73.4, 71.5, 71.3, 71.1, 68.8, 68.6, 67.3, 67.1, 62.9, 61.0, 57.0, 53.0, 45.5, 32.8, 23.0, 23.0, 20.9, 20.8, 20.8, 20.7, 20.6, 20.6; ESI-HRMS ($\text{C}_{33}\text{H}_{47}\text{NNaO}_{21}$): $[\text{M}+\text{Na}]^+$ m/z : 816.2538; found: 816.2487.

Methyl [methyl (4,7,8,9-tetro-*O*-acetyl-3,5-dideoxy-5-acetamido-*D*-glycero- α -*D*-gulo-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,4,6-tri-*O*-acetyl- β -*D*-galactopyranoside

(298): To a solution of **233** (30 mg, 0.03 mmol) in 6 mL of 1,4-dioxane/water (1:1) was added 30 mg of 5% Pd/C (100 wt%) followed by 0.1 mL of glacial acetic acid. The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h. Then the solution was filtered off and the solvents were evaporated. To the residue, 3 mL of Ac_2O and 3 mL of pyridine were added and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated and the residue was subjected to column chromatography on silica gel (5% MeOH in CH_2Cl_2) to afford the colorless oil **298** (19 mg; 77%). $[\alpha]_D^{21}$ -16.2 (c 0.5, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 5.88 (d, $J = 9.9$ Hz, 1H), 5.41 (d, $J = 7.1$ Hz, 1H), 5.04 (d, $J = 3.7$ Hz, 1H), 5.02 (td, $J = 5.9, 3.4$ Hz, 1H), 4.89 - 4.81 (m, 2H), 4.63 (dd, $J = 9.9, 3.5$ Hz, 1H), 4.40 (dd, $J = 9.4, 3.9$ Hz, 1H), 4.33 (d, $J = 7.9$ Hz, 1H), 4.22 (dd, $J = 11.8, 5.5$ Hz, 1H), 4.17-4.13 (m, 1H),

4.12-4.07 (m, 2H), 3.85 (s, 3H), 3.79 - 3.72 (m, 1H), 3.50 (s, 3H), 3.49 (d, $J = 7.0$ Hz, 1H), 2.40 (dd, $J = 13.3, 4.9$ Hz, 1H), 2.22 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.5, 170.4, 170.3, 170.3, 170.2, 169.9, 169.8, 169.7, 167.9, 101.9, 96.8, 73.1, 72.0, 71.7, 71.2, 70.5, 69.3, 67.5, 67.5, 61.9, 60.9, 57.0, 53.1, 45.4, 32.4, 23.1, 21.3, 20.7, 20.7, 20.6, 20.6, 20.5, 20.5; ESI-HRMS ($\text{C}_{33}\text{H}_{47}\text{NNaO}_{21}$): $[\text{M}+\text{Na}]^+$ m/z : 816.2538; found: 816.2483.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-acetamido- α -D-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 6)-2,3,4-tri-*O*-acetyl- β -D-galactopyranoside

(301): To a solution of **204** (100 mg, 0.12 mmol) in 14 mL of 1,4-dioxane/water (1:1) was added 100 mg of 5% Pd/C (100 wt%) followed by 0.36 mL of glacial acetic acid. The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h. Then the palladium catalyst was filtered off and the solvents were evaporated. To the residue, 7 mL of Ac_2O and 7 mL of pyridine were added and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated and the residue was subjected to column chromatography on silica gel (5% MeOH in CH_2Cl_2) to afford **301** (72 mg; 84%). $[\alpha]_{\text{D}}^{21}$ -11.6 (c 0.5, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 5.42 (d, $J = 3.3$ Hz, 1H), 5.36-5.31 (m, 1H), 5.26 (dd, $J = 8.9, 1.8$ Hz, 1H), 5.15 (dd, $J = 10.2, 8.0$ Hz, 1H), 5.05 - 5.00 (m, 1H), 4.86 - 4.79 (m, 1H), 4.43 (dd, $J = 7.9, 1.1$ Hz, 1H), 4.34 - 4.25 (m, 1H), 4.10-3.97 (m, 4H), 3.90 (t, $J = 6.8$ Hz, 1H), 3.77 (m, 1H), 3.76 (s, 3H), 3.51 (s, 3H), 3.42 - 3.35 (m, 1H), 2.50 (dd, $J = 12.9, 4.6$ Hz, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.86 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.9, 170.6, 170.3, 170.2, 170.1, 170.00, 169.7, 169.6,

167.9, 101.9, 98.9, 72.6, 71.4, 71.1, 69.1, 68.7, 68.1, 67.2, 67.1, 62.7, 62.6, 57.0, 52.9, 49.3, 37.9, 23.2, 21.0, 20.8, 20.8, 20.7, 20.7, 20.6, 20.5; ESI-HRMS ($C_{33}H_{47}NNaO_{21}$): $[M+Na]^+$ m/z: 816.2538; found: 816.2474.

Methyl [methyl(4,7,8,9-tetro-*O*-acetyl-3,5-dideoxy-5-acetamido-*D*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,4,6-tri-*O*-acetyl- β -*D*-galactopyranoside

(302): To a solution of **290** (90 mg, 0.11 mmol) in 12 mL of 1,4-dioxane:water (1:1) was added 90 mg of 5% Pd/C (100 wt%) followed by 0.29 mL of glacial acetic acid. The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h. Then the palladium catalyst was filtered off and the solvents were evaporated. To the residue, 6 mL of Ac_2O and 6 mL of pyridine were added and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated and the residue was subjected to column chromatography on silica gel (5% MeOH in CH_2Cl_2) to afford **302** (64 mg; 84%). $[\alpha]_D^{21}$ -12.2 (c 0.5, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$) δ 5.56 - 5.52 (m, 1H), 5.37 (dd, $J = 9.2, 2.7$ Hz, 1H), 5.08 (d, $J = 10.3$ Hz, 1H), 5.01 (dd, $J = 10.1, 8.0$ Hz, 1H), 4.91 (d, $J = 3.3$ Hz, 1H), 4.89 - 4.84 (m, 1H), 4.54 (dd, $J = 10.2, 3.4$ Hz, 1H), 4.50 (d, $J = 8.0$ Hz, 1H), 4.34 (dd, $J = 12.5, 2.7$ Hz, 1H), 4.09 - 3.97 (m, 4H), 3.85 (d, $J = 6.6$ Hz, 1H), 3.83 (s, 3H), 3.63 (dd, $J = 10.7, 2.7$ Hz, 1H), 3.51 (s, 3H), 2.57 (dd, $J = 12.7, 4.6$ Hz, 1H), 2.21 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9, 170.6, 170.5, 170.4, 170.3, 170.3, 169.7, 169.6, 167.9, 101.7, 96.0, 72.0, 71.4, 70.5, 69.6, 69.3, 67.8, 67.7, 67.0, 62.3, 62.0, 56.9, 53.2, 49.1, 37.5, 23.2, 21.5, 21.0, 20.8, 20.8, 20.7, 20.7, 20.6; ESI-HRMS ($C_{33}H_{47}NNaO_{21}$): $[M+Na]^+$ m/z: 816.2538; found: 816.2502.

Methyl [(3,5-dideoxy-5-acetamido-D-glycero- α -D-gulo-non-2-ulopyranosid)onic acid]-(2 \rightarrow 6)- β -D-galactopyranoside (299): To a solution of **297** (30 mg; 0.06 mmol) in 3 mL of H₂O was added 0.5 mL of saturated aq. Ba(OH)₂. The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and bubbled with CO₂, followed by filtering off the precipitate formed. The filtrate was then frozen using a dry ice-acetone bath and lyophilized to obtain the white foam **299** (16.8 mg, 92%). $[\alpha]_D^{21}$ -2.3 (c 0.5, H₂O); ¹H NMR (600 MHz, D₂O) δ 4.15 (dd, J = 9.7, 5.6 Hz, 1H), 3.87 (dd, J = 10.1, 8.3 Hz, 1H), 3.79 (d, J = 2.2 Hz, 1H), 3.76 (d, J = 3.0 Hz, 1H), 3.72 (t, J = 4.2 Hz, 1H), 3.68 (t, J = 3.5 Hz, 1H), 3.67 (m, 2H), 3.63 (d, J = 3.8 Hz, 1H), 3.53 (dd, J = 7.6, 2.5 Hz, 1H), 3.50 (d, J = 3.3 Hz, 1H), 3.48 - 3.45 (m, 1H), 3.43 (s, 3H), 3.37 - 3.30 (m, 1H), 2.36 (dd, J = 12.8, 4.6 Hz, 1H), 1.88 (s, 3H), 1.54 (t, J = 12.8 Hz, 1H); ¹³C NMR (151 MHz, D₂O) δ 174.7, 173.6, 103.9, 100.8, 73.5, 72.5, 72.2, 72.1, 70.5, 70.4, 68.8, 66.7, 63.9, 62.1, 57.4, 50.4, 35.5, 22.1; ESI-HRMS (C₁₈H₃₀NO₁₄): [M-H]⁻ m/z: 484.1666; found: 484.1736.

Methyl [(3,5-dideoxy-5-acetamido-D-glycero- α -D-gulo-non-2-ulopyranosid)onic acid]-(2 \rightarrow 3)- β -D-galactopyranoside (300): To a solution of **298** (10 mg; 0.02 mmol) in 2 mL of H₂O was added 0.2 mL of saturated aq. Ba(OH)₂. The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and bubbled with CO₂, followed by filtering off the precipitate formed. The filtrate was then frozen using a dry ice-acetone bath and lyophilized to obtain the white foam **300** (5.7 mg, 93%). $[\alpha]_D^{21}$ -1.6 (c 0.5, H₂O); ¹H NMR (600 MHz, D₂O) δ 4.21 (d, J = 8.0 Hz, 1H), 4.13 (d, J = 3.6 Hz, 1H), 4.00 (dd, J = 10.0, 2.9 Hz, 1H), 3.78 (d, J = 9.6 Hz, 1H), 3.68 (m, 4H), 3.57 (m, 3H), 3.53 - 3.46 (m, 2H), 3.41 (s, 3H), 2.42 (dd, J = 12.5, 4.4 Hz, 1H),

1.89 (s, 3H), 1.56 (t, $J = 12.5$ Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 174.7, 173.8, 103.6, 99.6, 75.3, 74.8, 72.2, 71.8, 70.9, 69.3, 66.8, 66.6, 61.9, 60.9, 60.0, 57.1, 50.2, 35.6, 22.0; ESI-HRMS ($\text{C}_{18}\text{H}_{30}\text{NO}_{14}$): $[\text{M}-\text{H}]^-$ m/z : 484.1666; found: 484.1697.

Methyl [(3,5-dideoxy-5-acetamido-D-glycero- α -D-gulo-non-2-ulopyranosid)onic acid]-(2 \rightarrow 3)- β -D-galactopyranoside (303): To a solution of **301** (40 mg; 0.08 mmol) in 4 mL of H_2O was added 0.7 mL of saturated aq. $\text{Ba}(\text{OH})_2$. The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and bubbled with CO_2 , followed by filtering off the precipitate formed. The filtrate was then frozen using a dry ice-acetone bath and lyophilized to obtain the white foam **303** (22.1 mg, 93%). $[\alpha]_D^{21}$ -1.4 (c 0.5, H_2O); ^1H NMR (600 MHz, D_2O) δ 4.14 (d, $J = 6.8$ Hz, 1H), 3.77 (dd, $J = 6.7, 2.1$ Hz, 1H), 3.71 (d, $J = 10.5$ Hz, 1H), 3.65 (t, $J = 9.9$ Hz, 1H), 3.61 (t, $J = 4.3$ Hz, 1H), 3.53 (m, 2H), 3.49 - 3.47 (m, 3H), 3.47 - 3.43 (m, 1H), 3.40 (s, 3H), 3.32 (t, $J = 8.4$ Hz, 1H), 2.56 (dd, $J = 12.4, 3.5$ Hz, 1H), 1.86 (s, 3H), 1.52 (t, $J = 12.0$ Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.0, 173.4, 103.8, 100.4, 73.3, 72.6, 72.5, 71.7, 70.6, 68.6, 68.2, 68.1, 63.3, 62.6, 57.3, 51.8, 40.1, 22.0%; ESI-HRMS ($\text{C}_{18}\text{H}_{30}\text{NO}_{14}$): $[\text{M}-\text{H}]^-$ m/z : 484.1666; found: 484.1692.

Methyl [(3,5-dideoxy-5-acetamido-D-glycero- α -D-galacto-non-2-ulopyranosid)onic acid]-(2 \rightarrow 3)- β -D-galactopyranoside (304): To a solution of **302** (50.0 mg; 0.1 mmol) in 5 mL of H_2O was added 0.8 mL of saturated aq. $\text{Ba}(\text{OH})_2$. The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and bubbled with CO_2 , followed by filtering off the precipitate formed. The filtrate was then frozen using a dry ice-acetone bath and lyophilized to obtain the white foam **304** (29.0 mg, 95%). $[\alpha]_D^{21}$ +0.3 (c 0.5, H_2O); ^1H NMR (600 MHz, D_2O) δ 4.21 (d, $J = 7.4$ Hz,

1H), 3.91 (dd, $J = 7.3$ Hz, 2.4 Hz, 1H), 3.77 (d, $J = 6.5$ Hz, 1H), 3.67 (m, 3H), 3.59 - 3.54 (m, 2H), 3.53 - 3.42 (m, 4H), 3.41 (s, 3H), 2.57 (d, $J = 12.1$ Hz, 1H), 1.85 (s, 3H), 1.62 (t, $J = 12.1$ Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 174.9, 173.8, 103.4, 99.8, 75.8, 74.8, 72.8, 71.7, 69.1, 68.2, 68.0, 67.5, 62.5, 60.9, 57.00, 51.6, 39.5, 22.0; ESI-HRMS ($\text{C}_{18}\text{H}_{30}\text{NO}_{14}$): $[\text{M}-\text{H}]^-$ m/z : 484.1666; found: 484.1717.

Methyl (1-Adamantanyl 5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-D-glycero- β -D-galacto-non-2-ulopyranosid)onate (318): A stirred solution of **160** (20 g, 31 mmol) in anhydrous methanol (500 mL) was treated with NaOMe (335 mg; 1.67 mmol) and stirred under argon at room temperature for 2 h, then quenched by addition of Amberlyst 15- H^+ ion exchange resin, filtered and concentrated to dryness. The residue was taken up in dry acetone (200 mL) under argon and treated with 2,2-dimethoxypropane (6.25 mL; 51 mmol) followed by camphor-10-sulfonic acid (250 mg; 1.08 mmol). The reaction mixture was stirred for 5 h and then quenched with triethylamine (0.5 mL). The solvents were evaporated and the residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated to dryness to give a residue that was purified by silica gel column chromatography eluting with 10% MeOH in CH_2Cl_2 to give **318** as a white solid (14.9 g; 93%), with ^1H and ^{13}C NMR consistent with the reported data. 143 ^1H NMR (600 MHz, CDCl_3) δ 6.68 (d, $J = 8.6$ Hz, 1H), 4.19 – 4.13 (m, 1H), 4.13 – 4.07 (m, 1H), 4.04 (d, $J = 10.5$ Hz, 1H), 4.01 – 3.94 (m, 2H), 3.76 (s, 3H), 3.44 (s, 1H), 2.49 (dd, $J = 13.4, 4.3$ Hz, 1H), 2.03 – 1.96 (m, 4H), 1.95 – 1.88 (m, 6H), 1.87 – 1.79 (m, 4H), 1.57 (s, 6H), 1.35 (s, 3H), 1.23 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.9, 171.1, 108.7, 74.1 72.2, 70.8, 67.3, 66.5, 53.1, 52.7, 52.3, 50.5, 50.0, 43.5, 43.0, 35.9, 29.7, 26.9, 26.7, 25.1, 23.1.

Methyl (1-Adamantanyl 5-acetamido-4-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate (319): To a stirred solution of **318** (14 g; 27.2 mmol) in a 1:7 mixture of pyridine and CH₂Cl₂ (140 mL) was added acetyl chloride (1.94 mL; 13.6 mmol) dropwise at -20 °C. After stirring for 1 h, the reaction mixture was quenched by addition of saturated aqueous NaHCO₃. The aqueous layer was extracted twice with CH₂Cl₂ and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and concentrated. The residue was purified by silica gel column chromatography eluting with 5% MeOH in CH₂Cl₂ to give **319** as an off-white solid (13.6 g; 90%). The ¹H and ¹³C NMR were consistent with the reported data.⁷ ¹H NMR (600 MHz, CDCl₃) δ 5.98 (t, *J* = 12.2 Hz, 1H), 5.42 – 5.31 (m, 1H), 4.26 – 4.20 (m, 1H), 4.18 – 4.10 (m, 4H), 4.07 – 4.02 (m, 1H), 3.98 – 3.90 (m, 1H), 3.78 (s, 3H), 3.46 (t, *J* = 6.8 Hz, 1H), 2.52 (dd, *J* = 13.5, 4.7 Hz, 1H), 2.04 – 2.00 (m, 4H), 1.96 (s, 8H), 1.92 – 1.87 (m, 3H), 1.67 – 1.56 (m, 6H), 1.37 (s, 3H), 1.26 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 172.2, 171.8, 170.1, 108.8, 85.9, 74.0, 72.6, 70.6, 68.5, 67.4, 52.7, 52.0, 50.5, 43.1, 40.3, 36.0, 29.8, 26.8, 25.2, 23.1, 21.0.

Methyl (1-adamantanyl 5-acetamido-4-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-7-*O*-(*tert*-butyldimethylsilyl)-2-thio-*D*-glycero- β -*D*-galacto-non-2-ulopyranosid)onate (320): To a stirred solution of **319** (6.2 g; 11.2 mmol) in dry CH₂Cl₂ (80 mL) at 0 °C were added DMAP (136 mg; 1.17 mmol) and Et₃N (7.78 mL; 55.8 mmol), followed after stirring at 0 °C for 10 min by TBSOTf (5.13 mL; 22.3 mmol). The resulting solution was brought to room temperature and stirred for 2 h, then washed with water and extracted with CH₂Cl₂. The organic layer was collected, dried over Na₂SO₄ and evaporated to dryness. The residue was subjected to column chromatography over silica gel eluting

with 45% EtOAc in hexanes to give **320** as off-white solid (6.65 g; 89%). $[\alpha]_D^{21} -74.3$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 5.35 (d, *J* = 9.1 Hz, 1H), 4.25 (d, *J* = 10.7 Hz, 1H), 4.21 (dd, *J* = 12.6, 6.3 Hz, 1H), 4.06 – 3.93 (m, 3H), 3.83 – 3.80 (m, 1H), 3.76 (s, 3H), 2.44 (dd, *J* = 13.6, 4.7 Hz, 1H), 2.00 (s, 6H), 1.90 (s, 3H), 1.64 (s, 6H), 1.39 (s, 3H), 1.28 (s, 3H), 0.89 (s, 8H), 0.84 (s, 9H), 0.17 (s, 3H), 0.09 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.9, 170.2, 169.7, 108.4, 85.8, 75.0, 73.5, 72.4, 70.2, 66.3, 52.5, 51.2, 50.2, 43.2, 39.6, 36.0, 29.8, 26.5, 26.2, 26.0, 25.7, 24.9, 23.4, 21.0, 18.4, 18.1, -3.0, -3.8, -3.9; ESI-HRMS (C₃₃H₅₅NO₉NaSiS) [M + Na]⁺ *m/z* 692.3265, found 692.3261.

Methyl (1-adamantanyl 5-acetamido-4-*O*-acetyl-7-*O*-(*tert*-butyldimethylsilyl)-3,5-dideoxy-9-*O*-(2,4,6-triisopropylbenzenesulfonyl)-2-thio-*D*-glycero-β-*D*-galacto-non-2-ulopyranosid)onate (322): To a stirred solution of **24** (2.5 g; 3.73 mmol) in dry CH₂Cl₂ (100 mL) at 0 °C was added 1:1 TFA:H₂O (5.5 mL) dropwise. The resulting mixture was stirred at 0 °C for 0.5 h, followed by 1 h at room temperature, then was quenched by addition of saturated aqueous NaHCO₃ (50 mL) and stirred for 15 min, and finally extracted with CH₂Cl₂. The combined organic phase was dried over Na₂SO₄ and evaporated to dryness to give the intermediate diol **321** as a white solid (2.4 g, 3.8 mmol), which was dissolved in dry pyridine (20 mL) and treated with 2,4,6-triisopropylbenzenesulfonyl chloride (8.1 g; 26.6 mmol). The resulting solution was stirred at room temperature for 16 h, then concentrated to dryness and the residue subjected to column chromatography over silica gel eluting with 45% EtOAc in hexanes to give **322** as a white solid (2.54 g; 76%). $[\alpha]_D^{21} -61.5$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.16 (s, 2H), 5.34 (dd, *J* = 9.9, 5.5 Hz, 1H), 5.24 (ddd, *J* = 11.1, 9.5, 4.7 Hz, 1H), 4.30 (t, *J* = 6.3 Hz, 5H), 4.20 – 4.06 (m, 3H), 3.96 (d, *J* = 5.1 Hz, 1H), 3.78 (s,

3H), 3.52 (br s, 1H), 2.93 – 2.84 (m, 1H), 2.43 (dd, $J = 12.7, 4.6$ Hz, 1H), 1.99 (s, 6H), 1.97 – 1.89 (m, 5H), 1.88 (s, 3H), 1.83 (d, $J = 10.8$ Hz, 3H), 1.64 (s, 6H), 1.30 – 1.19 (m, 18H), 0.84 (s, 9H), 0.12 (s, 3H), 0.03 (s, 3H): ^{13}C NMR (151 MHz, CDCl_3) δ 171.0, 170.3, 169.9, 153.7, 150.9, 129.0, 123.7, 85.8, 73.5, 71.8, 71.5, 70.7, 69.6, 52.8, 50.7, 50.3, 43.5, 39.6, 35.9, 34.2, 29.8, 29.6, 25.9, 24.8, 24.7, 23.5, 23.5, 23.5, 20.9, 18.2, -4.1, -4.9; ESI-HRMS ($\text{C}_{45}\text{H}_{73}\text{NO}_{11}\text{NaS}_2\text{Si}$) $[\text{M} + \text{Na}]^+$ m/z 918.4292, found 918.4292.

Methyl (1-adamantanyl 5-acetamido-4-*O*-acetyl-7-*O*-(*tert*-butyldimethylsilyl)-3,5-dideoxy-9-*O*-(2,4,6-triisopropylbenzenesulfonyl)-8-oxo-2-thio- β -D-galacto-non-2-

ulopyranosid)onate (323): To a stirred solution of **322** (2.4 g; 2.68 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C was added Dess-Martin periodinane (1.7 g; 4.02 mmol). The resulting mixture was brought to room temperature and stirred for 1 h then quenched with 20% aqueous $\text{Na}_2\text{S}_2\text{O}_3$ and extracted with CH_2Cl_2 . The organic layer was dried over Na_2SO_4 and evaporated to dryness, and the residue subjected to column chromatography over silica gel eluting with 40% EtOAc in hexanes to give **323** as a white solid (2.06 g; 86%). $[\alpha]_{\text{D}}^{21} -61.5$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.16 (s, 2H), 5.45 (d, $J = 13.3$ Hz, 1H), 5.38 (d, $J = 10.0$ Hz, 1H), 5.28 (ddd, $J = 12.0, 10.0, 4.6$ Hz, 3H), 4.66 (d, $J = 13.3$ Hz, 3H), 4.29 – 4.20 (m, 2H), 4.18 – 4.09 (m, 2H), 4.08 – 4.00 (m, 1H), 3.86 (s, 3H), 2.93 – 2.85 (m, 1H), 2.46 (dd, $J = 13.5, 4.6$ Hz, 1H), 2.04 – 1.98 (m, 2H), 1.97 (s, 3H), 1.88 (s, 3H), 1.86 – 1.80 (m, 5H), 1.70 – 1.56 (m, 7H), 1.28 – 1.14 (m, 18H), 0.77 (s, 9H), -0.05 (s, 3H), -0.32 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 203.6, 170.9, 170.3, 170.2, 153.8, 151.0, 128.6, 123.8, 86.0, 76.4, 74.5, 72.3, 69.9, 52.8, 50.8, 49.3, 43.2, 39.7, 36.0, 35.9, 34.3, 29.7, 29.7, 25.8, 25.7, 25.1, 24.4, 23.6, 23.5, 23.4, 20.9, 17.8, -4.5, -6.00; ESI-HRMS ($\text{C}_{45}\text{H}_{71}\text{NO}_{11}\text{NaS}_2\text{Si}$) $[\text{M} + \text{Na}]^+$ m/z 916.4136, found 916.4130.

Methyl (1-adamantanyl 5-acetamido-4-*O*-acetyl-7-*O*-(*tert*-butyldimethylsilyl)-3,5-dideoxy-9-*O*-(2,4,6-triisopropylbenzenesulfonyl)-2-thio-*L*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate (324): A solution of **323** (2.06 g; 2.31 mmol) in CH₂Cl₂ (60 mL) was cooled to -78 °C, and a solution of cerium (III) chloride heptahydrate (2.56 g; 6.91 mmol) in methanol (15 mL) was added. After 1 h, NaBH₄ (135 mg; 3.46 mmol) was added to the reaction mixture, which was then stirred at -78 °C for 2 h before it was quenched with saturated aqueous NH₄Cl, brought to room temperature and solvents were evaporated. The residue was taken up in EtOAc, washed with brine, and evaporated to dryness. The residue was subjected to column chromatography over silica gel eluting with 50% EtOAc in hexanes to give **324** as a white solid (1.82 g; 88%). [α]_D²¹ -83.4 (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.15 (s, 2H), 5.51 – 5.46 (m, 1H), 5.27 (ddd, *J* = 11.2, 9.3, 4.7 Hz, 1H), 4.27 – 4.20 (m, 2H), 4.20 – 4.05 (m, 5H), 4.04 – 3.98 (m, 1H), 3.97 (s, 1H), 3.76 (s, 3H), 2.94 – 2.84 (m, 1H), 2.41 (dd, *J* = 13.7, 4.7 Hz, 1H), 2.02 (s, 1H), 1.99 (s, 8H), 1.93 (d, *J* = 11.9 Hz, 1H), 1.89 (s, 3H), 1.84 (d, *J* = 11.5 Hz, 3H), 1.64 (s, 6H), 1.27 – 1.20 (m, 18H), 0.85 (s, 9H), 0.14 (s, 3H), 0.06 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 171.2, 170.5, 170.3, 153.6, 150.8, 129.2, 123.7, 85.6, 72.5, 71.4, 70.1, 69.7, 69.3, 60.4, 52.7, 51.7, 50.3, 43.5, 39.5, 36.0, 34.2, 29.8, 29.5, 25.9, 24.8, 24.7, 23.6, 23.5, 21.0, 20.9, 18.3, 14.2, -3.7, -5.1; ESI-HRMS (C₄₅H₇₃NO₁₁NaS₂Si) [M + Na]⁺ *m/z* 918.4292, found 918.4306.

Methyl (1-adamantanyl 5-acetamido-4-*O*-acetyl-7-*O*-(*tert*-butyldimethylsilyl)-3,5,9-tri-deoxy-9-iodo-2-thio-*L*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate (325): A stirred solution of **324** (1.8 g; 2.02 mmol) in dry acetone (25 mL) was treated with NaI (3 g; 20.2 mmol) and heated to 50 °C for 24 h. The solvent was evaporated and the residue

was subjected to column chromatography over silica gel eluting with 50% EtOAc in hexanes to give **325** as a white solid (1.29 g; 87%). $[\alpha]_D^{21} -52.7$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.20 (s, 2H), 5.48 (d, *J* = 9.0 Hz, 1H), 5.29 – 5.18 (m, 1H), 4.24 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.17 – 4.06 (m, 2H), 4.02 (t, *J* = 3.7 Hz, 1H), 3.91 (dd, *J* = 10.5, 5.8 Hz, 1H), 3.78 (s, 3H), 3.39 (dd, *J* = 10.2, 6.2 Hz, 1H), 3.34 (dd, *J* = 10.2, 5.9 Hz, 1H), 2.40 (dd, *J* = 13.7, 4.8 Hz, 1H), 1.99 (s, 6H), 1.96 – 1.90 (m, 1H), 1.90 (s, 3H), 1.85 (d, *J* = 10.6 Hz, 3H), 1.64 (s, 6H), 1.23 (t, *J* = 7.1 Hz, 1H), 0.90 (s, 9H), 0.18 (s, 3H), 0.13 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 171.0, 170.4, 170.1, 85.5, 73.6, 72.4, 70.6, 69.7, 60.4, 52.7, 51.6, 50.3, 43.6, 43.5, 39.5, 36.0, 29.9, 29.8, 26.1, 26.0, 23.6, 20.9, 18.3, 14.2, 11.1, -3.7, -4.7; ESI-HRMS (C₃₀H₅₀INO₈NaSSi) [M + Na]⁺ *m/z* 762.1969, found 762.1953.

Methyl (1-adamantanyl 5-acetamido-4-*O*-acetyl-7-*O*-(*tert*-butyldimethylsilyl)-3,5,9-tri-deoxy-2-thio-L-glycero- α -D-galacto-non-2-ulopyranosid)onate (326): A solution of **325** (1.29 g; 1.74 mmol) in EtOAc:Et₃N (10:1; 21 mL) was treated with 10% Pd/C (1.29 g; 100 wt%) and stirred under 1 atm of H₂ pressure for 1 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was subjected to column chromatography over silica gel eluting with 65% EtOAc in hexanes to give **326** as a colorless oil (1.07 g; 92%). $[\alpha]_D^{21} -88.3$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 5.45 (d, *J* = 8.9 Hz, 1H), 5.28 – 5.20 (m, 1H), 4.27 (dd, *J* = 10.6, 2.9 Hz, 1H), 4.12 (dd, *J* = 19.4, 9.7 Hz, 1H), 3.96 – 3.89 (m, 1H), 3.77 (s, 3H), 3.69 – 3.60 (m, 1H), 2.41 (dd, *J* = 13.7, 4.8 Hz, 1H), 2.00 (s, 9H), 1.96 – 1.91 (m, 1H), 1.89 (s, 3H), 1.84 (d, *J* = 10.8 Hz, 3H), 1.64 (s, 6H), 1.29 – 1.17 (m, 4H), 0.92 (s, 9H), 0.18 (s, 3H), 0.09 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.9, 170.4, 169.9, 85.5, 77.2, 72.9, 70.0, 67.3, 52.6, 51.5, 50.2,

43.4, 39.6, 36.0, 29.8, 29.3, 26.0, 23.6, 20.9, 20.6, 18.3, -3.8, -4.8; ESI-HRMS ($C_{30}H_{51}NO_8NaSSi$) $[M + Na]^+$ m/z 636.3002, found 636.3011.

Methyl (1-adamantanyl 5-acetamido-4,8-di-*O*-acetyl-7-*O*-(*tert*-butyldimethylsilyl)-3,5,9-tri-deoxy-2-thio-*L*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate (327): To a stirred solution of **326** (1.07 g; 1.73 mmol) in dry CH_2Cl_2 (30 mL) was added DMAP (22 mg; 0.18 mmol) followed by Et_3N (0.72 mL; 5.18 mmol). The resulting mixture was cooled to 0 °C and Ac_2O (0.24 mL; 2.60 mmol) was added dropwise. The resulting mixture was then brought to room temperature and stirred for 2 h before the solvent was evaporated to dryness and the residue purified by column chromatography over silica gel eluting with 50% EtOAc in hexanes to give **327** as a white solid (1.04 g; 91%). $[\alpha]_D^{21} - 113.0$ (c 1.0, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$) δ 5.56 (d, $J = 8.2$ Hz, 1H), 5.24 – 5.15 (m, 1H), 5.05 – 4.95 (m, 1H), 4.31 (dd, $J = 10.7, 2.1$ Hz, 1H), 4.02 – 3.96 (m, 1H), 3.84 (dd, $J = 7.8, 2.3$ Hz, 1H), 3.77 (s, 3H), 2.41 (dd, $J = 13.8, 4.6$ Hz, 1H), 2.01 (s, 6H), 1.98 (s, 6H), 1.97 – 1.91 (m, 1H), 1.90 (s, 3H), 1.84 (d, $J = 10.8$ Hz, 3H), 1.64 (s, 6H), 1.27 (d, $J = 6.4$ Hz, 3H), 0.90 (s, 9H), 0.18 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.7, 170.2, 169.9, 169.8, 85.7, 75.1, 72.7, 71.4, 70.5, 52.6, 51.4, 50.1, 43.4, 39.6, 36.0, 29.8, 25.9, 23.4, 21.6, 21.0, 18.2, 17.3, -4.3, -4.6; ESI-HRMS ($C_{32}H_{53}NO_9NaSSi$) $[M + Na]^+$ m/z 678.3108, found 678.3122.

Methyl (1-adamantanyl 4,8-di-*O*-acetyl-7-*O*-(*tert*-butyldimethylsilyl)-3,9-dideoxy-2-thio-*L*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate; Methyl (1-adamantanyl 4,8-di-*O*-acetyl-7-*O*-(*tert*-butyldimethylsilyl)-3,9-dideoxy-2-thio-*L*-glycero- α -*D*-gulo-non-2-ulopyranosid)onate (329): A solution of sialoside **327** (300 mg; 0.46 mmol) in dry dichloromethane (6 mL) was treated with dry pyridine (0.32 mL; 3.96 mmol) under argon

and cooled to -10 °C. After the solution was stirred for 15 min, crushed nitrosyl tetrafluoroborate (206 mg; 1.74 mmol) was added in one portion, after which the reaction mixture was stirred at -10 °C until TLC/MS showed complete conversion. The reaction mixture was diluted with cold dichloromethane (12 mL) and washed with cold 1 N HCl, saturated aqueous NaHCO₃ and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated to dryness below 10 °C to give the crude *N*-nitroso-sialoside, which was carried forward without any further purification. The crude *N*-nitroso-sialoside (315 mg; 0.46 mmol) was taken in levulinic acid (3 mL) and the resulting solution was heated to 50 °C for 6 h. After cooling to room temperature the reaction mixture was diluted with CH₂Cl₂ and quenched dropwise with saturated aqueous NaHCO₃. The aqueous layer was extracted with CH₂Cl₂ and the combined organic layer was dried over Na₂SO₄ and evaporated to dryness to give the crude 5-*O*-levulinyl derivative **328** as a yellowish oil (330 mg). To a solution of this crude levulinate **328** (330 mg; 0.46 mmol) in dry CH₂Cl₂ (10 mL) was added pyridine (1.03 mL) followed by hydrazine monohydrate (92 μL; 1.83 mmol) and acetic acid (0.79 mL). The resulting solution was stirred at room temperature for 1 h, then was quenched with saturated aqueous NH₄Cl and extracted with CH₂Cl₂. The organic layer was collected, dried over Na₂SO₄ and concentrated to dryness. The residue was subjected to column chromatography over silica gel eluting with 35% EtOAc in hexane to give **329** as a white solid (107 mg; 38% for 3 steps) in 1:1 mixture. $[\alpha]_D^{21} - 51.3$ (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.39 (d, *J* = 5.6 Hz, 1H), 5.24 – 5.15 (m, 2H), 5.14 – 5.08 (m, 1H), 4.49 (d, *J* = 6.8 Hz, 1H), 4.36 (dd, *J* = 10.0, 3.5 Hz, 1H), 3.95 (t, *J* = 4.6 Hz, 1H), 3.91 (dd, *J* = 5.2, 3.6 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.75 – 3.68 (m, 2H), 3.61 (s, 1H), 2.86 (d, *J* = 3.0 Hz, 1H), 2.75 (s, 1H), 2.70 (s, 1H),

2.54 (dd, $J = 13.2, 5.0$ Hz, 9H), 2.44 (dd, $J = 13.6, 5.6$ Hz, 9H), 2.08 (s, 3H), 2.07 (s, 3H), 2.06 – 2.04 (m, 3H), 1.97 (s, 3H), 1.91 – 1.83 (m, 6H), 1.71 (d, $J = 2.1$ Hz, 1H), 1.67 (s, 9H), 1.34 (d, $J = 6.4$ Hz, 3H), 1.29 (d, $J = 6.6$ Hz, 3H), 1.25 (s, 3H), 0.92 (s, 9H), 0.90 (s, 9H), 0.21 (s, 3H), 0.13 (s, 3H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.6, 170.4, 170.3, 170.2, 170.0, 89.8, 86.9, 85.8, 75.3, 74.4, 73.3, 72.9, 72.1, 71.8, 71.0, 70.7, 70.5, 52.6, 50.0, 45.9, 43.6, 43.4, 38.8, 36.0, 29.8, 25.8, 25.7, 21.5, 21.4, 21.1, 20.9, 18.1, 18.0, 17.8, 16.4, -4.3, -4.4, -4.6, -4.9; ESI-HRMS ($\text{C}_{30}\text{H}_{50}\text{O}_9\text{NaSSi}$)[$\text{M} + \text{Na}$] $^+$ m/z 637.2843, found 637.2833.

Methyl (1-Adamantanyl 5-acetamido-4-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-7-*O*-(2-naphthyl)methyl-2-thio- β -D-galacto-non-2-ulopyranosid)onate

(330): To a stirred solution of **319** (5.76 g; 10.37 mmol) in dry DMF (55 mL) under argon was added 2-(bromomethyl)naphthalene (5.04 g; 17.66 mmol). The resulting mixture was stirred at -10 °C for 15 min. Then NaH (60% mineral oil; 767 mg; 11.77 mmol) was added to it at -10 °C. The resulting mixture was brought to 0 °C and stirred for 3 h, then quenched with H_2O , diluted with EtOAc and washed with water and brine. The organic layer was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to column chromatography over silica gel with 50% EtOAc in hexanes to obtain **330** an off-white solid (5.91 g; 82%). $[\alpha]_{\text{D}}^{21} -66.7$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.85 (s, 1H), 7.80 – 7.75 (m, 3H), 7.59 (d, $J = 8.4$ Hz, 1H), 7.48 – 7.40 (m, 2H), 5.45 – 5.32 (m, 1H), 5.25 (d, $J = 9.5$ Hz, 1H), 5.02 – 4.83 (m, 2H), 4.38 – 4.26 (m, 2H), 4.26 – 4.07 (m, 3H), 3.94 – 3.91 (m, 1H), 3.78 (s, 3H), 2.47 (dd, $J = 13.4, 4.7$ Hz, 1H), 2.03 – 1.94 (m, 8H), 1.92 – 1.83 (m, 4H), 1.80 (s, 3H), 1.63 (s, 6H), 1.47 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.8, 170.1, 170.0,

136.0, 133.2, 132.9, 128.0, 127.9, 127.6, 127.1, 126.6, 125.9, 125.8, 108.0, 86.0, 76.9, 76.4, 74.6, 73.0, 69.3, 65.8, 52.6, 50.6, 50.1, 43.3, 39.6, 36.0, 29.8, 26.5, 24.4, 23.3, 21.0; ESI-HRMS (C₃₈H₄₉NO₉NaS) [M + Na]⁺ *m/z* 718.3026, found 718.3011.

Methyl (1-Adamantanyl 4-*O*-acetyl-3,5-dideoxy-5-en-8,9-*O*-isopropylidene-7-*O*-(2-naphthyl)methyl-2-thio-β-D-ribo-non-2-ulopyranosid)onate (332), and, Methyl (1-Adamantanyl 4-*O*-acetyl-3-deoxy-8,9-*O*-isopropylidene-5-*O*-levulinoyl-7-*O*-(2-naphthyl)methyl-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (333): A solution of **330** (5.7 g; 8.17 mmol) in dry dichloromethane (60 mL) was treated with dry pyridine (6.65 mL; 81.7 mmol) under argon and cooled to -10 °C. After stirring for 15 min, crushed nitrosyl tetrafluoroborate (3.8 g; 32.8 mmol) was added in one portion to the solution after which stirring was continued at -10 °C until completion of the reaction (TLC/MS). The reaction mixture was diluted with cold dichloromethane (120 mL) and washed with cold 1 N HCl, saturated aq NaHCO₃ and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated below 10 °C to obtain the *N*-nitrosyl derivative **331**, which was carried forward without further purification. To a solution of the crude nitrosated sialoside intermediate **331** (5.94 g; 6.7 mmol) in dry dichloromethane (70 mL) at -10 °C was added a mixture of cold solution of 18-crown-6 (3.9 g; 14.8 mmol) and freshly prepared sodium 2,2,2-trifluoroethoxide (1.64 g; 13.4 mmol) in dry dichloromethane (35 mL). After stirring for 5 min, the reaction mixture was treated with a cold solution of levulinic acid (15.6 g; 134.3 mmol) in dry dichloromethane (70 mL) and stirred at -10 °C for 10 min, then warmed to 0 °C, quenched with saturated aqueous NaHCO₃, diluted with dichloromethane, washed with cold brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column

chromatography over silica gel eluting with 4% acetone in toluene to afford **332** (1.1 g; 21%) and 6% acetone in toluene to afford **333** (3.4 g; 55%) as off-white solids. **332**: $[\alpha]_D^{21} -12.3$ (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.86 – 7.78 (m, 4H), 7.53 – 7.43 (m, 3H), 5.29 – 5.24 (m, 1H), 5.21 (d, *J* = 5.3 Hz, 1H), 5.01 (t, *J* = 11.7 Hz, 1H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.53 (dd, *J* = 11.0, 6.2 Hz, 1H), 4.10 (d, *J* = 6.5 Hz, 1H), 4.10 (d, *J* = 6.5 Hz, 1H), 4.04 (d, *J* = 4.5 Hz, 1H), 3.80 (d, *J* = 6.5 Hz, 1H), 3.76 (s, 3H), 2.65 – 2.59 (m, 1H), 2.25 (dd, *J* = 12.7, 4.7 Hz, 1H), 2.04 – 1.94 (m, 10H), 1.62 (br s, 7H), 1.45 (s, 3H), 1.39 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.1, 170.0, 154.2, 135.7, 132.2, 132.9, 127.9, 127.8, 127.6, 126.4, 126.0, 126.0, 125.7, 109.4, 96.8, 83.9, 78.4, 76.3, 72.0, 65.3, 62.5, 52.4, 50.9, 43.8, 43.3, 36.7, 36.0, 29.9, 29.8, 26.5, 25.5, 21.2; ESI-HRMS (C₃₆H₄₄O₈NaS) [M + Na]⁺ *m/z* 659.2655, found 659.2641. **333**: $[\alpha]_D^{21} -66.9$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.86 – 7.74 (m, 4H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.47 – 7.39 (m, 2H), 5.45 – 5.38 (m, 1H), 5.24 (t, *J* = 9.6 Hz, 1H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.67 (d, *J* = 10.9 Hz, 1H), 4.46 (dd, *J* = 9.8, 1.7 Hz, 1H), 4.40 – 4.35 (m, 1H), 4.26 – 4.22 (m, 1H), 4.17 (dd, *J* = 8.5, 7.2 Hz, 1H), 3.99 (d, *J* = 2.0 Hz, 1H), 3.76 (s, 3H), 2.68 – 2.62 (m, 2H), 2.53 (t, *J* = 2.0 Hz, 1H), 2.51 (d, *J* = 4.9 Hz, 1H), 2.49 – 2.45 (m, 2H), 2.34 (s, 1H), 2.14 (s, 3H), 2.07 – 1.96 (m, 7H), 1.93 – 1.86 (m, 4H), 1.65 (s, 6H), 1.48 (s, 3H), 1.30 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 206.0, 171.5, 170.3, 169.8, 135.8, 133.2, 132.9, 129.0, 128.2, 128.0, 127.8, 127.6, 127.0, 126.6, 125.8, 125.7, 108.0, 85.9, 76.8, 75.9, 74.7, 72.3, 69.5, 69.3, 65.5, 52.5, 50.2, 43.3, 39.3, 37.6, 36.1, 36.0, 29.9, 29.7, 27.9, 26.5, 24.3, 20.9; ESI-HRMS (C₄₁H₅₂O₁₁NaS) [M + Na]⁺ *m/z* 775.3128, found 775.3109.

Methyl (1-Adamantanyl 4-*O*-acetyl-3-deoxy-5-*O*-levulinoyl-7-*O*-(2-naphthyl)methyl-9-*O*-(2,4,6-triisopropylbenzenesulfonyl)-2-thio-*D*-glycero- α -*D*-galacto-non-2-

ulopyranosid)onate (335): To a stirred solution of **333** (3.2 g; 4.2 mmol) in dichloromethane (170 mL) at 0 °C was added 1:1 v/v TFA:H₂O mixture (8.2 mL) dropwise. The resulting mixture was stirred at 0 °C for 0.5 h and then brought to room temperature. The reaction mixture was further stirred for 1 h at room temperature and then quenched with saturated aqueous NaHCO₃. The reaction mixture was diluted with dichloromethane and washed with brine, dried (Na₂SO₄) and evaporated to dryness to give the crude 8,9-diol derivative **334** as a white solid (3.03 g, 4.3 mmol) that was taken up in dry dichloromethane:triethylamine (88 mL; 10:1) under argon and treated with Bu₂SnO (0.38 g; 1.3 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCI) (2.57 g; 8.5 mmol). The resulting mixture was stirred at room temperature for 10 h, then was filtered and evaporated to dryness. The residue was subjected to column chromatography over silica gel eluting with 40% EtOAc in hexanes to give **335** as a white solid (3.45 g; 83%). [α]_D²¹ -48.2 (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.98 – 7.67 (m, 4H), 7.53 – 7.35 (m, 3H), 7.18 (s, 2H), 5.44 – 5.34 (m, 1H), 5.24 (t, *J* = 9.4 Hz, 1H), 4.81 (d, *J* = 11.0 Hz, 1H), 4.70 (d, *J* = 11.0 Hz, 1H), 4.65 (dd, *J* = 9.8, 2.9 Hz, 1H), 4.52 (dd, *J* = 10.5, 2.3 Hz, 1H), 4.40 – 4.32 (m, 1H), 4.31 – 4.25 (m, 1H), 4.24 – 4.11 (m, 2H), 3.90 – 3.79 (m, 1H), 3.76 (s, 3H), 2.96 – 2.84 (m, 1H), 2.59 – 2.44 (m, 2H), 2.45 – 2.29 (m, 2H), 2.28 – 2.16 (m, 1H), 2.00 (s, 6H), 1.91 (s, 6H), 1.77 (d, *J* = 10.1 Hz, 3H), 1.69 – 1.46 (m, 6H), 1.35 – 1.16 (m, 18H); ¹³C NMR (151 MHz, CDCl₃) δ 206.1, 171.6, 170.3, 170.1, 153.7, 150.9, 134.9, 133.1, 133.0, 129.2, 128.1, 128.0, 127.6, 127.3, 126.4, 126.0, 126.0, 123.7, 85.6, 76.9, 73.9, 71.0, 70.8, 69.1, 68.8, 52.8, 50.4, 43.3, 39.2, 37.5, 35.9, 34.2,

29.7, 29.7, 29.5, 27.9, 24.8, 24.7, 23.5, 20.8; ESI-HRMS ($C_{53}H_{70}O_{13}NaS_2$) $[M + Na]^+$ m/z 1001.4156, found 1001.4144.

Methyl (1-Adamantanyl 4-*O*-acetyl-3-deoxy-5-*O*-levulinoyl-7-*O*-(2-naphthyl)methyl-9-*O*-(2,4,6-triisopropylbenzenesulfonyl)-2-thio-L-glycero- α -D-galacto-non-2-

ulopyranosid)onate (338): To a stirred solution of **335** (3.35 g; 3.42 mmol) in dry dichloromethane (60 mL) under argon at 0 °C was added dry pyridine (2.2 mL; 27.4 mmol) followed by Tf_2O (2.3 mL; 13.7 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1 h then was diluted with dichloromethane and washed sequentially with 1 N HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was collected, dried over Na_2SO_4 and evaporated to dryness to get the triflated derivative **337**. To the intermediate **337** in dry acetonitrile (60 mL) under argon was added tetrabutylammonium nitrite (5.94 g; 20.5 mmol). The reaction mixture was stirred at room temperature for 10 h, then was diluted with EtOAc and washed with water. The organic layer was collected, dried (Na_2SO_4) and concentrated under vacuum and the residue purified by column chromatography over silica gel eluting with 40% EtOAc in hexanes to afford **338** as a white solid (2.4 g; 72%). $[\alpha]_D^{21} -74.6$ (c 1.0, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$) δ 7.87 – 7.73 (m, 4H), 7.52 – 7.39 (m, 3H), 7.18 (s, 2H), 5.48 – 5.36 (m, 1H), 5.34 (t, $J = 9.4$ Hz, 1H), 4.85 (d, $J = 11.1$ Hz, 1H), 4.75 (d, $J = 11.1$ Hz, 1H), 4.66 (dd, $J = 9.7, 3.8$ Hz, 1H), 4.33 – 4.21 (m, 2H), 4.19 – 4.06 (m, 3H), 3.89 – 3.81 (m, 1H), 3.74 (s, 3H), 3.35 (br s, 1H), 2.89 (dt, $J = 13.7, 6.8$ Hz, 1H), 2.69 – 2.58 (m, 1H), 2.57 – 2.42 (m, 3H), 2.42 – 2.34 (m, 1H), 2.06 (s, 3H), 2.01 (s, 3H), 1.98 – 1.85 (m, 6H), 1.77 (d, $J = 11.6$ Hz, 3H), 1.64 – 1.47 (m, 6H), 1.34 – 1.17 (m, 18H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.1, 172.2, 170.2, 153.7, 150.8, 134.9, 133.2, 133.1, 129.1, 128.2, 128.0, 127.6, 127.1, 126.1, 126.1,

126.0, 123.8, 85.5, 75.7, 74.6, 70.7, 70.2, 69.5, 69.4, 69.2, 52.7, 50.4, 43.2, 39.1, 37.6, 35.9, 34.2, 29.7, 29.7, 29.6, 28.0, 24.7, 24.7, 23.5, 20.9; ESI-HRMS ($C_{53}H_{70}O_{13}NaS_2$) [$M + Na$]⁺ m/z 1001.4156, found 1001.4153.

Methyl (1-Adamantanyl 4-O-acetyl-3-deoxy-9-iodo-5-O-levulinoyl-7-O-(2-naphthyl)methyl-2-thio-L-glycero- α -D-galacto-non-2-ulopyranosid)onate (339): To a solution of **338** (2.2 g; 2.24 mmol) in dry acetone (35 mL) was added NaI (3.35 g; 22.43 mmol). The resulting mixture was heated to reflux for 22 h then evaporated to dryness and the residue purified by column chromatography over silica gel eluting with 40% EtOAc in hexanes to give **339** as a colorless oil (1.5 g; 81%). $[\alpha]_D^{21} -82.1$ (c 1.0, $CHCl_3$): ¹H NMR (600 MHz, $CDCl_3$) δ 7.91 – 7.75 (m, 4H), 7.47 (dt, $J = 10.3, 7.5$ Hz, 3H), 5.47 – 5.33 (m, 1H), 5.28 (t, $J = 9.4$ Hz, 1H), 4.93 (t, $J = 11.5$ Hz, 1H), 4.79 (d, $J = 11.5$ Hz, 1H), 4.65 (dd, $J = 9.8, 4.1$ Hz, 1H), 4.00 (dd, $J = 10.2, 5.3$ Hz, 1H), 3.90 (t, $J = 3.9$ Hz, 1H), 3.78 (s, 3H), 3.47 (dd, $J = 10.1, 6.3$ Hz, 1H), 3.41 (dd, $J = 10.1, 6.1$ Hz, 1H), 3.27 (s, 1H), 2.74 (d, $J = 6.7$ Hz, 1H), 2.66 – 2.56 (m, 1H), 2.55 – 2.47 (m, 2H), 2.47 – 2.40 (m, 1H), 2.40 – 2.32 (m, 1H), 2.06 (s, 3H), 2.01 (s, 3H), 1.94 – 1.87 (m, 6H), 1.78 (d, $J = 11.6$ Hz, 3H), 1.69 – 1.50 (m, 6H); ¹³C NMR (151 MHz, $CDCl_3$) δ 206.1, 172.0, 170.4, 170.1, 85.5, 77.8, 74.5, 71.0, 70.3, 69.9, 69.1, 52.8, 50.4, 43.3, 39.2, 37.6, 35.9, 29.8, 29.7, 29.6, 28.0, 20.9, 10.3; ESI-HRMS ($C_{38}H_{47}O_{10}NaSI$) [$M + Na$]⁺ m/z 845.1832, found 845.1839.

Methyl (1-Adamantanyl 4-O-acetyl-3,9-dideoxy-5-O-levulinoyl-7-O-(2-naphthyl)methyl-2-thio-L-glycero- α -D-galacto-non-2-ulopyranosid)onate (340): To a stirred solution of **339** (1.5 g; 1.82 mmol) in EtOAc:Et₃N (33 mL; 10:1) was added 10% Pd/C (1.5 g; 100 wt%). The resulting mixture was stirred under 1 atm of hydrogen for 1 h then filtered and concentrated under vacuum. The residue was subjected to column

chromatography over silica gel eluting with 45% EtOAc in hexanes to obtain **340** as a white solid (1.16 g; 91%). $[\alpha]_D^{21} -61.1$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.92 – 7.72 (m, 4H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.49 – 7.40 (m, 2H), 5.41 (ddd, *J* = 11.5, 9.0, 4.9 Hz, 1H), 5.29 (t, *J* = 9.4 Hz, 1H), 4.91 (d, *J* = 11.4 Hz, 1H), 4.77 (d, *J* = 11.4 Hz, 1H), 4.63 (dd, *J* = 9.9, 3.4 Hz, 1H), 4.08 (dt, *J* = 10.5, 5.8 Hz, 1H), 3.78 (s, 3H), 3.48 (t, *J* = 3.7 Hz, 1H), 2.92 (d, *J* = 4.4 Hz, 1H), 2.67 – 2.36 (m, 5H), 2.07 (s, 3H), 2.00 (s, 3H), 1.99 – 1.89 (m, 7H), 1.83 (d, *J* = 11.8 Hz, 3H), 1.59 (s, 6H), 1.32 (d, *J* = 6.4 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 206.1, 171.9, 170.3, 170.1, 135.6, 133.2, 133.0, 128.1, 127.9, 127.6, 126.7, 126.1, 126.0, 125.9, 85.5, 81.2, 74.6, 70.9, 70.3, 69.3, 67.5, 52.7, 50.3, 43.3, 39.2, 37.6, 35.9, 29.8, 29.6, 28.0, 20.9, 20.7; ESI-HRMS (C₃₈H₄₈O₁₀NaS) [M + Na]⁺ *m/z* 719.2866, found 719.2849.

Methyl (1-Adamantanyl 4,8-di-O-acetyl-3,9-dideoxy-5-O-levulinoyl-7-O-(2-naphthyl)methyl-2-thio-L-glycero-α-D-galacto-non-2-ulopyranosid)onate (341): To a stirred solution of **340** (1.1 g; 1.57 mmol) in dry dichloromethane (25 mL) was added DMAP (19 mg; 0.16 mmol) followed by Et₃N (0.66 mL; 4.72 mmol). The resulting mixture was cooled to 0 °C and Ac₂O (0.23 mL; 2.37 mmol) was added. The resulting mixture was brought to room temperature and stirred for 3 h then concentrated to dryness and the residue subjected to column chromatography over silica gel eluting with 40% EtOAc in hexanes to give **341** as a colorless oil (1.11 g; 95%). $[\alpha]_D^{21} -66.3$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.90 – 7.73 (m, 4H), 7.55 – 7.46 (m, 1H), 7.49 – 7.40 (m, 2H), 5.37 (ddd, *J* = 11.3, 8.6, 4.8 Hz, 9H), 5.24 – 5.11 (m, 1H), 4.91 (d, *J* = 11.5 Hz, 1H), 4.78 (d, *J* = 11.5 Hz, 1H), 4.57 (dd, *J* = 9.8 Hz, 3.9 Hz, 1H), 3.78 (s, 3H), 3.60 (dd, *J* = 6.0, 3.9 Hz, 7H), 2.73 – 2.66 (m, 1H), 2.62 – 2.41 (m, 5H), 2.10 (s, 3H), 2.00 (s,

3H), 2.00 (s, 3H), 1.99 – 1.83 (m, 9H), 1.69 – 1.51 (m, 7H), 1.35 (d, $J = 6.4$ Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 206.1, 171.7, 170.3, 170.2, 170.1, 135.7, 133.2, 132.9, 127.9, 127.9, 127.6, 126.7, 126.1, 126.0, 125.8, 85.4, 79.3, 74.4, 70.3, 70.1, 69.5, 52.7, 50.3, 43.5, 39.1, 37.6, 36.0, 29.8, 29.7, 28.0, 21.3, 20.9, 16.8; ESI-HRMS ($\text{C}_{40}\text{H}_{50}\text{O}_{11}\text{NaS}$) [$\text{M} + \text{Na}$] $^+$ m/z 761.2977, found 761.2972.

Methyl (1-Adamantanyl 4,8-di-*O*-acetyl-3,9-dideoxy-5-*O*-levulinoyl-2-thio-L-glycero- α -D-galacto-non-2-ulopyranosid)onate (342): A stirred solution of **341** (1.06 g; 1.4 mmol) in dichloromethane:H₂O (105 mL; 20:1) was treated with DDQ (0.64 g; 5.62 mmol) and stirred at room temperature for 4 h before it was diluted with dichloromethane (100 mL) and washed with saturated aqueous NaHCO₃ (90 mL X 3). The organic layer was collected, dried over Na₂SO₄ and concentrated under vacuum. The residue was subjected to column chromatography over silica gel eluting with 75% EtOAc in hexanes to give **342** as a white solid (0.67 g; 78%). $[\alpha]_D^{21} -76.5$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 5.38 (ddd, $J = 11.3, 9.0, 4.8$ Hz, 1H), 5.12 (t, $J = 9.3$ Hz, 1H), 5.05 – 4.95 (m, 1H), 4.27 (dd, $J = 9.7, 1.9$ Hz, 1H), 3.80 (s, 3H), 3.51 (t, $J = 6.4$ Hz, 1H), 2.81 – 2.66 (m, 3H), 2.61 – 2.50 (m, 3H), 2.16 (s, 3H), 2.09 (s, 3H), 2.01 (s, 3H), 2.00 – 1.94 (m, 5H), 1.84 (d, $J = 11.8$ Hz, 3H), 1.75 (s, 2H), 1.64 (s, 6H), 1.32 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 206.1, 171.9, 170.7, 170.1, 170.1, 85.5, 71.9, 71.5, 70.6, 69.9, 68.9, 52.8, 50.4, 43.5, 39.2, 37.7, 36.0, 29.8, 29.7, 27.9, 21.3, 20.9, 17.2; ESI-HRMS ($\text{C}_{29}\text{H}_{42}\text{O}_{11}\text{NaS}$) [$\text{M} + \text{Na}$] $^+$ m/z 621.2346, found 621.2343.

Methyl (1-Adamantanyl 4,8-di-*O*-acetyl-3,9-dideoxy-2-thio-L-glycero- α -D-galacto-non-2-ulopyranosid)onate (312): To a stirred solution of **342** (600 mg; 0.99 mmol) in dry dichloromethane (30 mL) was added pyridine (2.8 mL) and hydrazine monohydrate

(1.4 mL; 3.96 mmol) followed by glacial acetic acid (2.0 mL). The resulting solution was stirred at room temperature for 1 h then diluted with saturated aqueous NH_4Cl and extracted with dichloromethane. The organic layer was collected, dried (Na_2SO_4) and evaporated to dryness. The residue was subjected to column chromatography over silica gel eluting with 80% EtOAc in hexanes to obtain **312** as a colorless oil (437 mg; 87%). $[\alpha]_D^{21}$ -61.1 (c 1.0, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 5.24 – 5.16 (m, 1H), 5.12 (dt, $J = 10.5, 5.2$ Hz, 1H), 4.17 (d, $J = 9.4$ Hz, 6H), 3.83 (s, 1H), 3.79 (s, 3H), 3.70 (t, $J = 8.2$ Hz, 1H), 2.91 (s, 1H), 2.81 (s, 1H), 2.55 (dd, $J = 13.6, 4.8$ Hz, 1H), 2.10 (s, 3H), 2.06 (s, 3H), 2.03 – 1.93 (m, 5H), 1.90 – 1.80 (m, 4H), 1.72 (s, 2H), 1.64 (s, 5H), 1.32 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.4, 170.9, 170.6, 85.6, 73.4, 72.7, 72.4, 70.9, 70.1, 52.7, 50.3, 43.5, 39.1, 36.0, 29.8, 21.4, 21.1, 17.3; ESI-HRMS ($\text{C}_{24}\text{H}_{36}\text{O}_9\text{NaS}$) $[\text{M} + \text{Na}]^+$ m/z 523.1978, found 523.1982.

Methyl (1-Adamantanyl 4,8-di-*O*-acetyl-5,7-di-azido-3,5,7,9-tetra-deoxy-2-thio-L-glycero- α -L-manno-non-2-ulopyranosid)onate (311): A stirred solution of **312** (400 mg; 0.8 mmol) in dry dichloromethane (25 mL) at 0 °C was treated with pyridine (0.76 mL; 9.6 mmol) followed by dropwise addition of Tf_2O (0.8 mL; 4.8 mmol). The resulting mixture was stirred at 0 °C for 1 h then was diluted with dichloromethane and washed with 1 N HCl, saturated aqueous NaHCO_3 and brine solution. The organic layer was collected, dried (Na_2SO_4) and evaporated to dryness and the residue taken up in dry DMF (25 mL) at 0 °C and treated with NaN_3 (2.05 g; 31.99 mmol). The resulting mixture was stirred at 0 °C for 24 h then was diluted with EtOAc, and washed with water. The organic layer was collected, dried (Na_2SO_4) and evaporated to dryness. The residue was subjected to column chromatography over silica gel eluting with 20% EtOAc in hexanes to give

311 as a white solid (307 mg; 70%). $[\alpha]_D^{21} -33.1$ (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.33 (tdd, *J* = 12.7, 5.7, 2.7 Hz, 1H), 4.16 – 4.07 (m, 2H), 4.02 (dd, *J* = 9.8, 2.2 Hz, 1H), 3.82 (s, 3H), 2.33 – 2.26 (m, 2H), 2.11 (s, 3H), 2.09 (s, 3H), 2.05 – 1.92 (m, 5H), 1.81 (d, *J* = 11.7 Hz, 3H), 1.65 (s, 4H), 1.56 (s, 3H), 1.32 (d, *J* = 6.3 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 169.8 (X 3), 86.1, 77.6, 77.2, 70.4, 69.2, 69.1, 65.1, 59.1, 52.8, 50.6, 43.6, 35.9, 34.7, 29.8, 29.7, 21.1, 20.7, 14.2; ESI-HRMS (C₂₄H₃₄N₆O₇NaS) [M + Na]⁺ *m/z* 573.2107, found 573.2104.

General Coupling Protocol with Donor 311: A mixture of donor **311** (50 mg; 0.09 mmol), acceptor (0.11 mmol), and activated 4Å acid-washed powdered molecular sieves (200 mg; 2 g/mmol of donor) in CH₂Cl₂/CH₃CN (2:1, 2.0 mL) was stirred for 2 h at room temperature under argon then cooled to -78 °C, and treated with *N*-iodosuccinimide (24 mg; 0.11 mmol) and TfOH (2 μL; 0.013 mmol). The reaction mixture was stirred at -78 °C for 7 h and then quenched with triethylamine (10 μL). The mixture was diluted with CH₂Cl₂, filtered through Celite, washed with 20% aqueous Na₂S₂O₃, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel eluting with mixtures of EtOAc and hexanes to afford the coupled products.

Methyl [Benzyl (4,8-di-*O*-acetyl-5,7-di-azido-3,5,7,9-tetra-deoxy-L-glycero-β-L-manno-non-2-ulopyranosid)onate] (43): Glycoside **343** was prepared according to the general glycosylation procedure from donor **311** (50 mg; 0.09 mmol) and benzyl alcohol (12 μL; 0.11 mmol) in CH₂Cl₂/CH₃CN (2.1 mL, 2:1) at -78 °C. After chromatographic purification over silica gel eluting with 25% EtOAc in hexanes, **343** was obtained as a colorless oil (37.9 mg; 89%). $[\alpha]_D^{21} -36.3$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ

7.43 – 7.26 (m, 5H), 5.44 (qd, $J = 6.4, 2.6$ Hz, 1H), 4.97 – 4.88 (m, 1H), 4.76 (d, $J = 11.5$ Hz, 1H), 4.43 (d, $J = 11.5$ Hz, 1H), 4.09 – 4.04 (m, 2H), 3.71 (s, 3H), 3.64 – 3.61 (m, 1H), 2.47 (dd, $J = 12.7, 4.5$ Hz, 1H), 2.26 (t, $J = 12.8$ Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 1.38 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 169.9, 168.5, 136.6, 128.3, 127.9, 127.8, 71.6, 70.7, 69.3, 66.7, 63.9, 58.2, 52.8, 32.3, 29.7, 21.0, 20.6, 12.9; ESI-HRMS ($\text{C}_{21}\text{H}_{26}\text{N}_6\text{O}_8\text{Na}$) $[\text{M} + \text{Na}]^+$ m/z 513.1710, found 513.1718.

Methyl [Methyl (4,8-di-*O*-acetyl-5,7-di-azido-3,5,7,9-tetra-deoxy-L-glycero- β -L-manno-non-2-ulopyranosid)onate]-(2 \rightarrow 6)-2,3,4-tri-*O*-benzyl- β -D-galactopyranoside (344):

Glycoside **344** was prepared according to the general glycosylation procedure from donor **311** (50 mg; 0.09 mmol) and methyl 2,3,4-tribenzyl- β -D-galactopyranoside **187** as acceptor (50.3 mg; 0.11 mmol) in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (2.1 mL, 2:1) at -78 °C. After chromatographic purification over silica gel eluting with 25% EtOAc in hexanes, **344** was obtained as a white foam (63.8 mg; 83%). $[\alpha]_D^{21} -23.1$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.63 – 6.98 (m, 15H), 5.34 (qd, $J = 6.4, 2.5$ Hz, 1H), 4.97 (t, $J = 9.6$ Hz, 1H), 4.92 – 4.84 (m, 2H), 4.78 – 4.70 (m, 3H), 4.61 (d, $J = 11.5$ Hz, 1H), 4.29 (d, $J = 7.6$ Hz, 1H), 4.04 (s, 1H), 3.97 (dd, $J = 9.9, 2.1$ Hz, 1H), 3.88 (d, $J = 2.6$ Hz, 1H), 3.85 – 3.75 (m, 2H), 3.59 (s, 3H), 3.57 – 3.49 (m, 6H), 2.40 (dd, $J = 12.7, 4.5$ Hz, 1H), 2.21 – 2.14 (m, 2H), 2.13 (s, 3H), 2.07 (s, 3H), 1.33 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 169.7, 169.5, 167.9, 138.9, 138.6, 128.3, 128.2, 128.0, 128.0, 127.6, 127.4, 127.4, 127.2, 104.9, 99.3, 81.9, 79.5, 75.0, 74.3, 73.3, 72.9, 72.5, 71.6, 70.6, 69.3, 63.8, 62.5, 58.1, 57.1, 52.8, 32.1, 29.7, 21.00, 20.6, 12.9; ESI-HRMS ($\text{C}_{42}\text{H}_{50}\text{N}_6\text{O}_{13}\text{Na}$) $[\text{M} + \text{Na}]^+$ m/z 869.3334, found 869.3327.

Methyl [Methyl (4,8-di-*O*-acetyl-5,7-di-azido-3,5,7,9-tetra-deoxy-L-glycero- β -L-manno-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,4,6-tri-*O*-benzyl- β -D-galactopyranoside

(345): Glycoside **345** was prepared according to the general glycosylation procedure from donor **311** (60 mg; 0.11 mmol) and methyl 2,4,6-tribenzyl- β -D-galactopyranoside **188** as acceptor (60.4 mg; 0.13 mmol) in CH₂Cl₂/CH₃CN (2.4 mL, 2:1) at -78 °C. After chromatographic purification over silica gel eluting with 25% EtOAc in hexanes, **345** was obtained as a white foam (71 mg; 77%). $[\alpha]_D^{21}$ -32.3 (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.80 – 6.92 (m, 15H), 5.50 – 5.32 (m, 1H), 4.94 (ddd, *J* = 11.4, 5.9, 4.3 Hz, 2H), 4.84 (d, *J* = 10.9 Hz, 1H), 4.64 (d, *J* = 10.9 Hz, 1H), 4.59 – 4.49 (m, 3H), 4.33 (d, *J* = 7.7 Hz, 1H), 4.01 – 3.92 (m, 3H), 3.86 (d, *J* = 3.0 Hz, 1H), 3.73 (s, 3H), 3.72 – 3.60 (m, 4H), 3.57 (s, 3H), 3.38 (dd, *J* = 9.9, 1.4 Hz, 1H), 2.41 (t, *J* = 12.9 Hz, 1H), 2.30 (dd, *J* = 13.5, 5.1 Hz, 1H), 2.05 (s, 3H), 2.00 (s, 3H), 1.29 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 169.7, 169.4, 167.9, 139.1, 138.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.5, 127.5, 127.2, 104.8, 100.4, 77.6, 77.5, 76.3, 75.2, 74.8, 74.0, 73.6, 71.5, 70.2, 69.7, 69.5, 63.5, 57.9, 57.1, 53.0, 29.7, 21.0, 20.6, 12.7; ESI-HRMS (C₄₂H₅₀N₆O₁₃Na) [M + Na]⁺ *m/z* 869.3334, found 869.3348.

Methyl [Methyl (4,8-di-*O*-acetyl-5,7-di-azido-3,5,7,9-tetra-deoxy-L-glycero- β -L-manno-non-2-ulopyranosid)onate]-(2 \rightarrow 4)-2,3,6-tri-*O*-benzyl- β -D-galactopyranoside

(346): Glycoside **346** was prepared according to the general glycosylation procedure using donor **311** (50 mg; 0.09 mmol) and methyl 2,3,6-tribenzyl- β -D-galactopyranoside **230** as acceptor (50.3 mg; 0.11 mmol) in CH₂Cl₂/CH₃CN (2.1 mL, 2:1) at -78 °C. After chromatographic purification over silica gel eluting with 25% EtOAc in hexanes, **346** was obtained as a white foam (40.6 mg; 53%). $[\alpha]_D^{21}$ -16.7 (*c* 1.0, CHCl₃); ¹H NMR (400

MHz, CDCl₃) δ 7.39 – 7.31 (m, 10H), 7.23 – 7.14 (m, 5H), 5.52 (dd, J = 6.4, 1.8 Hz, 1H), 5.40 – 5.32 (m, 1H), 4.84 (d, J = 10.8 Hz, 1H), 4.78 (d, J = 12.5 Hz, 1H), 4.67 (d, J = 7.1 Hz, 1H), 4.64 (d, J = 7.9 Hz, 1H), 4.47 (d, J = 11.6 Hz, 1H), 4.41 (d, J = 10.8 Hz, 1H), 4.22 (d, J = 7.6 Hz, 1H), 4.13 – 3.88 (m, 5H), 3.81 (t, J = 10.8 Hz, 1H), 3.63 – 3.54 (m, 2H), 3.52 (s, 3H), 3.46 (s, 3H), 3.41 (dd, J = 10.0, 2.5 Hz, 1H), 2.37 (dd, J = 12.9, 3.5 Hz, 1H), 2.14 (s, 3H), 2.08 (s, 3H), 1.21 (d, J = 6.4 Hz, 3H); ¹³C NMR (101 MHz, d₂o) δ 169.8, 169.3, 167.7, 138.4, 137.6, 136.8, 128.5, 128.4, 128.1, 128.0, 128.0, 127.9, 127.4, 127.3, 105.2, 98.8, 81.4, 78.2, 76.6, 75.2, 73.4, 72.2, 70.4, 68.4, 67.1, 66.1, 64.7, 58.8, 57.1, 52.0, 33.3, 29.6, 20.9, 20.5, 13.5; ESI-HRMS (C₄₂H₅₀N₆O₁₃Na) [M + Na]⁺ m/z 869.3334, found 573.3338.

Methyl [Methyl (7-acetamido-4,8-di-*O*-acetyl-5-azido-3,5,7,9-tetra-deoxy-L-glycero- β -L-manno-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,4,6-tri-*O*-benzyl- β -D-galactopyranoside (349): To a stirred solution of **345** (20 mg; 0.024 mmol) in dry CHCl₃ (1.0 mL) were added 2,6-lutidine (0.3 mL) and thioacetic acid (0.3 mL, 166.7 mmol). The resulting mixture was heated at 60 °C for 10 h then concentrated under vacuum and the residue subjected to column chromatography over silica gel eluting with 65% EtOAc in hexanes to give **349** as a colorless oil (13.9 mg; 68%). [α]_D²¹ -38.2 (*c* 0.8, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.48 – 7.26 (m, 15H), 5.60 (d, J = 9.8 Hz, 1H), 5.34 – 5.21 (m, 1H), 5.01 – 4.89 (m, 2H), 4.84 (d, J = 11.0 Hz, 1H), 4.72 – 4.61 (m, 2H), 4.60 – 4.46 (m, 3H), 4.32 (d, J = 7.7 Hz, 1H), 4.01 (dd, J = 10.0, 3.1 Hz, 1H), 3.87 (d, J = 3.0 Hz, 1H), 3.82 – 3.74 (m, 2H), 3.72 (s, 3H), 3.66 – 3.60 (m, 2H), 3.56 (s, 3H), 2.47 (t, J = 13.0 Hz, 1H), 2.26 (dd, J = 13.4, 4.6 Hz, 1H), 2.16 – 2.04 (m, 2H), 2.03 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.25 (d, J = 6.3 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.0,

169.9, 169.8, 168.2, 139.1, 138.5, 138.3, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.4, 127.4, 127.1, 104.8, 100.4, 77.6, 77.6, 76.0, 75.1, 74.8, 73.7, 73.4, 71.9, 69.9, 69.4, 68.9, 58.3, 57.0, 52.8, 51.4, 47.9, 29.7, 23.5, 21.1, 20.6, 14.1; ESI-HRMS (C₄₄H₅₄N₄O₁₄Na) [M + Na]⁺ *m/z* 885.3534, found 885.3546.

Methyl [Methyl (7-acetamido-4,8-di-*O*-acetyl-3,5,7,9-tetra-deoxy-5-(1,1-dimethylethoxy)carbonylamino-L-glycero-β-L-manno-non-2-ulopyranosid)onate]-(2→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (350): To a stirred solution of **349** (10 mg; 0.012 mmol) in pyridine (1 mL) were added H₂O (104 μL; 5.78 mmol), Et₃N (25 μL; 0.18 mmol) and 1,3-propanedithiol (24 μL; 0.24 mmol). The resulting mixture was stirred at room temperature for 2 h then concentrated under vacuum and the residue subjected to column chromatography over silica gel eluting with 10% MeOH in CH₂Cl₂ as solvent to afford the crude 5-amino derivative as a white solid (7.6 mg). This solid (7.6 mg; 0.008 mmol) was dissolved in 1,4-dioxane (0.5 mL) and treated with Et₃N (5 μL; 0.03 mmol) and Boc₂O (3.5 μL; 0.015 mmol). The resulting mixture was heated at 60 °C for 10 h then concentrated under vacuum and the residue subjected to column chromatography over silica gel eluting with 75% EtOAc in hexanes to give **350** as a colorless oil (7.8 mg; 72%): [α]²¹_D -26.3 (*c* 0.4, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.52 – 7.20 (m, 15H), 5.31 – 5.23 (m, 2H), 4.90 (d, *J* = 11.4 Hz, 1H), 4.87 – 4.81 (m, 2H), 4.80 – 4.74 (m, 1H), 4.66 (d, *J* = 11.0 Hz, 1H), 4.56 (dd, *J* = 11.1, 6.2 Hz, 2H), 4.53 – 4.46 (m, 2H), 4.31 (d, *J* = 7.7 Hz, 1H), 4.16 (d, *J* = 9.3 Hz, 1H), 3.96 (dd, *J* = 9.9, 2.9 Hz, 1H), 3.91 – 3.87 (m, 1H), 3.70 (s, 3H), 3.68 – 3.61 (m, 3H), 3.55 (s, 3H), 2.34 (dd, *J* = 13.4, 4.5 Hz, 1H), 2.10 – 1.98 (m, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.92 (s, 3H), 1.43 (s, 9H), 1.26 (d, *J* = 6.6 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.3, 170.1, 169.4, 167.9,

155.7, 139.1, 138.3, 128.4, 128.3, 128.3, 128.00, 127.8, 127.6, 127.5, 127.1, 104.7, 100.6, 80.0, 77.6, 76.0, 75.2, 74.9, 73.5, 72.5, 69.6, 69.3, 68.1, 57.0, 52.9, 50.2, 47.9, 45.9, 30.3, 28.5, 28.2, 23.3, 21.1, 20.7, 14.0; ESI-HRMS (C₄₉H₆₄N₂O₁₆Na) [M + Na]⁺ *m/z* 959.415, found 959.4161.

Methyl [(7-acetamido-3,5,7,9-tetra-deoxy-5-(1,1-dimethylethoxy)carbonylamino-L-glycero-β-L-manno-non-2-ulopyranosid)onate]-(2→3)-β-D-galactopyranoside (351):

A solution of **350** (6.0 mg; 0.0064 mmol) in 1:1 aqueous Ba(OH)₂:1,4-dioxane (0.6 mL) was heated at 60 °C for 1 h then cooled to room temperature saturated with CO₂ and filtered. The filtrate was evaporated under vacuum and the residue taken up in 1:1 H₂O:1,4-dioxane (0.6 mL) then treated with 10% Pd/C (6.0 mg; 100 wt%) and stirred under 1 atm of hydrogen for 16 h. The reaction mixture was filtered and the filtrate was lyophilization to give **351** as a white foam (2.9 mg; 80%). [α]²¹_D -8.3 (*c* 0.2, D₂O); ¹H NMR (600 MHz, D₂O) δ 4.37 (d, *J* = 8.0 Hz, 1H), 4.10 (dt, *J* = 12.7, 6.2 Hz, 1H), 4.05 (dd, *J* = 9.9, 3.2 Hz, 1H), 4.01 – 3.96 (m, 1H), 3.91 (d, *J* = 2.9 Hz, 1H), 3.86 (d, *J* = 2.9 Hz, 1H), 3.81 – 3.75 (m, 2H), 3.74 – 3.67 (m, 3H), 3.66 – 3.61 (m, 2H), 3.57 (s, 3H), 3.55 – 3.48 (m, 2H), 2.53 – 2.42 (m, 1H), 2.20 – 2.11 (m, 1H), 2.01 (s, 1H), 1.97 (s, 3H), 1.80 (s, 1H), 1.63 (t, *J* = 12.6 Hz, 1H), 1.56 – 1.49 (m, 1H), 1.41 (s, 9H), 1.36 – 1.29 (m, 1H), 1.19 (d, *J* = 6.9 Hz, 3H); ¹³C NMR (151 MHz, D₂O) δ 173.6, 172.9, 157.4, 103.6, 100.3, 80.3, 75.5, 74.9, 73.6, 71.6, 69.3, 69.1, 67.1, 66.6, 61.0, 60.4, 57.1, 53.6, 49.3, 35.3, 27.7, 22.1, 16.7; ESI-HRMS (C₃₈H₄₈O₁₀NaS) [M - H]⁻ *m/z* 567.2401, found 567.2411.

Methyl [Benzyl (5,7-di-acetamido-4,8-di-O-acetyl-3,5,7,9-tetra-deoxy-L-glycero-β-L-manno-non-2-ulopyranosid)onate] (347): To a stirred solution of glycoside **343** (20 mg; 0.04 mmol) in dry pyridine (1.0 mL) was added AcSH (1.0 mL). The resulting

mixture was stirred at room temperature for 40 h then concentrated to dryness and the residue subjected to column chromatography over silica gel eluting with 8% MeOH in CH₂Cl₂ to give **347** as a colorless oil (15.6 mg; 73%). $[\alpha]_D^{21}$ -42.1 (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.48 – 7.24 (m, 5H), 5.81 (d, *J* = 9.4 Hz, 1H), 5.71 (d, *J* = 10.7 Hz, 1H), 5.37 – 5.25 (m, 1H), 4.84 (d, *J* = 11.3 Hz, 1H), 4.82 – 4.76 (m, 1H), 4.58 – 4.47 (m, 2H), 4.41 (d, *J* = 11.3 Hz, 1H), 4.01 (dd, *J* = 10.0, 1.8 Hz, 1H), 3.72 (s, 3H), 2.49 (dd, *J* = 13.2, 4.5 Hz, 1H), 2.03 (s, 3H), 1.98 (s, 3H), 1.98 (s, 3H), 1.90 – 1.87 (m, 1H), 1.35 (d, *J* = 6.7 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.9, 170.6, 170.5, 169.9, 168.4, 136.5, 128.4, 128.1, 128.0, 99.2, 72.4, 70.0, 67.7, 66.9, 52.7, 50.5, 45.3, 33.2, 29.7, 23.3, 23.3, 21.2, 20.9, 14.1; ESI-HRMS (C₂₅H₃₄N₂O₁₀Na) [M + Na]⁺ *m/z* 545.2111, found 545.2127.

5,7-Di-acetamido-3,5,7,9-tetradecoxy-L-glycero-α-L-manno-2-nonulosonic Acid - Pse5Ac7Ac (2): A stirred solution of **347** (10.0 mg; 0.02 mmol) in 1:1 aqueous Ba(OH)₂:1,4-dioxane (1.0 mL) was heated at 60 °C for 1 h then cooled to room temperature, saturated with CO₂ and filtered. The filtrate was evaporated to dryness and then taken up in 1:1 H₂O:1,4-dioxane (1.0 mL), treated with 10% Pd/C (10.0 mg; 100 wt%) and stirred under 1 atm of hydrogen for 14 h. The solution was filtered and the filtrate was subjected to lyophilization to give **2** as a white foam (5.2 mg; 81%) whose ¹H and ¹³C NMR data were consistent with the literature.^{43,97,140,141} ¹H NMR (600 MHz, D₂O) δ 4.25 (br s, 1H), 4.21 – 4.11 (m, 3H), 4.04 (d, *J* = 10.4 Hz, 1H), 2.02 (s, 3H), 1.99 (s, 3H), 1.95 (dd, *J* = 13.3, 4.8 Hz, 1H), 1.80 (t, *J* = 12.7 Hz, 1H), 1.12 (d, *J* = 6.4 Hz, 3H); ¹³C NMR (151 MHz, D₂O) δ 176.3, 174.7, 173.9, 96.3, 70.0, 66.8, 65.3, 52.9, 48.8, 34.8, 22.0, 21.9, 15.1.

Methyl [Methyl (4,8-di-*O*-acetyl-5-azido-3,5,7,9-tetra-deoxy-7-(1,1-dimethylethoxy)carbonylamino-L-glycero-β-L-manno-non-2-ulopyranosid)onate]-(2→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (352): To a stirred solution of **345** (15 mg; 0.018 mmol) in pyridine (1.0 mL) at 0 °C were added H₂O (0.16 mL; 8.88 mmol), Et₃N (37 μL; 0.266 mmol) and 1,3-propanedithiol (9 μL; 0.09 mmol). The resulting solution was stirred at 0 °C for 5 h, then concentrated under vacuum and the residue subjected to column chromatography over silica gel eluting with 75% EtOAc in hexanes to give a white fluffy solid (8 mg) which was taken to the next step without any further characterization. To a solution of the above solid (8 mg; 0.01 mmol) in 1,4-dioxane (1.0 mL) were added Et₃N (27 μL; 0.19 mmol) and Boc₂O (21.2 mg; 0.1 mmol). The resulting mixture was heated at 60 °C for 10 h then concentrated under vacuum and the residue was subjected to column chromatography over silica gel eluting with 35% EtOAc in hexanes to give **351** as a colorless oil (8.3 mg; 51%). $[\alpha]^{21}_{\text{D}} -24.3$ (*c* 0.5, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.59 – 7.04 (m, 15H), 5.33 (d, *J* = 6.4 Hz, 1H), 4.87 (d, *J* = 11.4 Hz, 1H), 4.81 – 4.76 (m, 2H), 4.64 (dd, *J* = 6.5, 4.4 Hz, 1H), 4.57 (d, *J* = 10.4 Hz, 1H), 4.49 (d, *J* = 11.2 Hz, 2H), 4.45 (d, *J* = 11.9 Hz, 1H), 4.36 – 4.27 (m, 3H), 3.92 (d, *J* = 7.1 Hz, 1H), 3.79 – 3.72 (m, 2H), 3.69 (s, 3H), 3.68 – 3.58 (m, 4H), 3.55 (s, 3H), 3.43 – 3.32 (m, 2H), 2.42 (d, *J* = 9.1 Hz, 1H), 2.03 (s, 3H), 1.94 (s, 3H), 1.47 (s, 9H), 1.26 (d, *J* = 6.4 Hz, 1H); ¹³C NMR (151 MHz, CDCl₃) δ 169.9, 169.7, 167.8, 155.3, 138.8, 138.5, 138.0, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.3, 104.7, 99.9, 80.4, 77.4, 76.4, 76.3, 75.0, 74.8, 73.5, 73.3, 72.1, 70.3, 68.8, 67.6, 63.9, 57.1, 53.0, 46.3, 30.9, 29.7, 28.3, 21.1, 20.7, 12.8; ESI-HRMS (C₄₇H₆₀N₄O₁₅Na) [M + Na]⁺ *m/z* 943.3953, found 943.3975.

Methyl [(5-acetamido-4,8-di-*O*-acetyl-3,5,7,9-tetra-deoxy-7-*N*-(1,1-dimethylethoxy)carbonylamino-*L*-glycero- β -*L*-manno-non-2-ulopyranosid)onate]-(2 \rightarrow 3)-2,4,6-tri-*O*-benzyl- β -*D*-galactopyranoside (353): A stirred solution of **352** (8.0 mg; 0.009 mmol) in dry pyridine (0.5 mL) was treated with AcSH (0.5 mL), after 15 h at room temperature with further pyridine (0.5 mL) and AcSH (0.5 mL), and after another 15 at room temperature with pyridine (0.5 mL) and AcSH (0.5 mL). Finally, stirring was continued for 15 h (total 45 h) at room temperature before the solvents were evaporated and the residue was subjected to column chromatography over silica gel eluting with 75% EtOAc in hexanes to give **353** as a colorless oil (5.8 mg; 71%). $[\alpha]_D^{21} -22.1$ (*c* 0.4, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.46 – 7.17 (m, 15H), 5.31 – 5.24 (m, 1H), 5.22 (d, *J* = 10.4 Hz, 1H), 4.90 (d, *J* = 11.4 Hz, 1H), 4.87 – 4.72 (m, 2H), 4.66 (d, *J* = 11.0 Hz, 1H), 4.59 – 4.53 (m, 2H), 4.52 – 4.45 (m, 2H), 4.30 (d, *J* = 7.7 Hz, 1H), 4.16 (d, *J* = 10.0 Hz, 1H), 3.95 (d, *J* = 9.6 Hz, 1H), 3.87 (s, 1H), 3.70 (s, 3H), 3.68 – 3.61 (m, 2H), 3.55 (s, 3H), 2.41 – 2.28 (m, 2H), 1.95 (s, 3H), 1.92 (s, 3H), 1.92 (s, 3H), 1.43 (s, 9H), 1.26 (d, *J* = 6.7 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 170.3, 170.1, 169.4, 167.9, 155.8, 139.1, 138.3, 130.8, 128.8, 128.4, 128.3, 128.0, 127.8, 127.7, 127.5, 127.2, 104.7, 100.6, 80.0, 77.6, 76.0, 75.2, 74.9, 73.5, 72.5, 69.6, 69.3, 68.1, 68.1, 57.1, 52.9, 50.2, 45.9, 31.9, 28.2, 22.7, 21.2, 20.6, 19.8, 14.1; ESI-HRMS (C₄₉H₆₄N₂O₁₆Na) [M + Na]⁺ *m/z* 959.4154, found 959.4142.

Methyl [(5-acetamido-3,5,7,9-tetra-deoxy-7-*N*-(1,1-dimethylethoxy)carbonylamino-*L*-glycero- β -*L*-manno-non-2-ulopyranosid)onate]-(2 \rightarrow 3)- β -*D*-galactopyranoside (354): A stirred solution of **353** (4.0 mg; 0.0043 mmol) in 1:1 aq. Ba(OH)₂ and 1,4-dioxane (0.4 mL) was heated to 60 °C for 1 h, then cooled to room temperature, saturated

with CO₂, and filtered. The filtrate was evaporated in vacuum and then taken up in 1:1 H₂O:1,4-dioxane (0.4 mL) and treated with 10% Pd/C (4.0 mg; 100 wt%). The resulting solution was stirred under 1 atm of hydrogen for 16 h, then filtered and lyophilized to give **354** as a white foam (2.0 mg; 83%). $[\alpha]_D^{21} -4.8$ (*c* 0.13, D₂O); ¹H NMR (600 MHz, D₂O) δ 4.37 (d, *J* = 8.0 Hz, 1H), 4.13 – 4.07 (m, 1H), 4.04 (d, *J* = 9.9 Hz, 1H), 3.97 (dd, *J* = 13.3, 8.1 Hz, 1H), 3.91 (s, 1H), 3.85 (s, 1H), 3.81 – 3.75 (m, 2H), 3.74 – 3.68 (m, 3H), 3.67 – 3.62 (m, 2H), 3.56 (s, 3H), 3.53 – 3.47 (m, 2H), 3.43 (dd, *J* = 11.9, 5.6 Hz, 1H), 2.53 – 2.45 (m, 1H), 2.15 (s, 1H), 2.05 (s, 1H), 1.96 (s, 3H), 1.62 (t, *J* = 12.4 Hz, 1H), 1.53 (br s, 1H), 1.40 (s, 9H), 1.31 (d, *J* = 6.9 Hz, 1H), 1.19 (d, *J* = 6.3 Hz, 3H); ¹³C NMR (151 MHz, D₂O) δ 173.6, 173.0, 157.2, 103.5, 100.2, 80.2, 75.4, 74.8, 73.5, 69.2, 68.9, 67.0, 66.5, 65.5, 60.9, 57.1, 53.6, 49.2, 45.4, 45.3, 35.3, 23.2, 22.0, 16.5; ESI-HRMS (C₃₈H₄₈O₁₀NaS) [M - H]⁻ *m/z* 567.2401, found 567.2415.

REFERENCES

1. Schauer, R. *Sialic Acids : Chemistry, Metabolism and Function*; Springer Verlag : New York, 1982; Vol 10.
2. Inoue, Y.; Lee, Y. C.; Troy, F. A. *Sialobiology and other Novel Forms of Glycosylation*, II Eds,; Gakushin : Osaka, Japan, **1999**.
3. Rosenberg, A. *Biology of the Sialic Acids*; Plenum : New York, **1995**.
4. Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754-2794.
5. Simanek, E. E.; McGarvey, G. J.; Jablonwski, J. A.; Wong, C.H. *Chem. Rev.* **1998**, *98*, 833-862.
6. Schwarzkopt, M.; Knobloch K. P.; Rohde, E. *Proc. Natl. Acad. Sci. USA* **2002**; *99*, 5267-5270.
7. Varki, A. *Nature*, **2007**, *446*, 1023-1029.
8. Herrler, G.; Rott, R., Klenk, H.D. *EMBO J.* **1985**, *4*, 1503-1506.
9. Schauer, R. *Glycoconjugate J.* **2000**, *7*, 485-499.
10. Angata, T.; Varki, A. *Chem Rev.* **2002**, *102*, 439-469.
11. Ilver, D.; Johansson, P.; Miller-Podraza H. *Methods Enzymol.* **2003**, *363*, 134-157.
12. Strasser, P.; Unger, U.; Strobl, B. *Glycoconj. J.* **2004**, *20*, 551-561.
13. Sharon, N. *Biochim. Biophys. Acta.* **2006**, *1760*, 527-537.
14. Lehmann, F.; Tiralongo, E.; Tiralongo, J. *Cell Mol. Life Sci.* **2006**, *63*, 1331-1354.
15. Suzuki, Y.; Ito, T. Suzuki, T. *J. Virol.* **2000**, *74*, 11825-11831.

16. Gagneux, P.; Cheriyan, M.; Hurtado-Ziola, N. *J. Biol. Chem.* **2003**, *278*, 48245-48250.
17. Klein, A.; Krishna, M.; Varki, N. M. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7782-7786.
18. Martin, M. J.; Rayner, J. C.; Gagneux P. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 12819-12824.
19. Glode, M. P.; Sutton, A.; Robbins, J. B.; McCracken, G.H.; Gotschlich, E. C.; Kaijser, B.; Hanson, L. A. *J. Infect. Dis.* **1977**, *136 Suppl.*, S93-S97.
20. Yuki, N. *Cell. Mol. Life. Sci.* **2000**, *57*, 527-533.
21. Miller-Podraza, H.; Milh, M. A.; Bergstrom, J.; Karlsson, K. A. *Glycoconjugate J.* **1996**, *13*, 453-460.
22. Miller-Podraza, H.; Bergstrom, J.; Milh, M. A.; Karlsson, K. A. *Glycoconjugate J.* **1997**, *14*, 467-471.
23. Ono, E.; Abe, K.; Nakazawa, M.; Naiki, M. *Infect. Immun.* **1989**, *57*, 907-911.
24. Smit, H.; Gastra, W.; Kamerling, J. P.; Vliegthart, J. F. G.; de Graaf, F. K. *Infect. Immun.* **1984**, *46*, 578-584.
25. Yuyama, Y.; Yoshimatsu, K.; Ono, E.; Saito, M.; Naiki, M. *J. Biochem. (Tokyo)* **1993**, *113*, 488-492.
26. Richards, R. L.; Moss, J.; Alving, C. R.; Fishman, P. H.; Brady, R. O. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 1673-1676.
27. Knirel, Y. A.; Shashkov, A. S.; Tsvetkov, Y. E.; Jansson, P. -E.; Zaehring, U. *Adv. Carbohydr. Chem. Biochem.* **2003**, *58*, 371-417.
28. Zunk, M.; Kiefel, M. J. *RSC Adv.* **2014**, *4*, 3413-3421.

29. Miyagi, T.; Yamaguchi, K. *In Comprehensive Glycoscience Kamerling, J. P. Ed. Elsevier Amsterdam* **2007**, *3*, 297-323.
30. McNaught, A. D. *Carbohydr. Res.* **1997**, *297*, 1-92.
31. Knirel, Y. A.; Rietschel, E. T.; Marre, R.; Zahringer, U. *Eur. J. Biochem.* **1994**, *221*, 239-245.
32. Cazalet, C.; Jarraud, S.; Ghavi-Helm, Y.; Kunst, F.; Glaser, P.; Etienne, J.; Buchreiser, C. *Genome Res.* **2008**, *18*, 431-441.
33. Logan, S. M.; Hui, J. P. M.; Vinogradov, E.; Aubry, A. J.; Melanson, J. E.; Kelly, J. F.; Nothaft, H.; Soo, E. C. *FEBS J.* **2009**, *276*, 1014-1023.
34. McNally, D. J.; Aubry, A. J.; Hui, J. P. M.; Khieu, N. H.; Whitfield, D.; Ewing, C. P.; Guerry, P.; Brisson, J. -R.; Logan, S. M.; Soo, E. C. *J. Biol. Chem.* **2007**, *282*, 14463-14475.
35. Kodali, S.; Vinogradov, E.; Lin, F.; Khoury, N.; Hao, L.; Pavliak, V.; Jones, H.; Laverde, C. D.; Heubner, J.; Jansen, K. U., Anderson, A. S., Donald, R. G. K. *J. Biol. Chem.* **2015**, *290*, 19512-19526.
36. Kenyon, J. J.; Marzaioli, A. M.; De Castro, C.; Hall, R. M. *Glycobiology* **2015**, *25*, 644-654.
37. Morrison, M. J.; Imperiali, B. *Biochemistry* **2014**, *53*, 624-628.
38. Vinogradov, E.; St Michael, F.; Cox, A. D. *Carbohydr. Res.* **2017**, *440-441*, 10-15.
39. Zunk, M.; Williams, J.; Carter, J.; Kiefel, M. J. *Org. Bio. Chem.* **2014**, *12*, 2918-2925.
40. Watson, D. C.; Leclerc, S.; Wakarchuk, W. W.; Young, N. M. *Glycobiology* **2011**, *21*, 99-108.

41. Watson, D. C.; Wakarchuk, W. W.; Gervais, C.; Durocher, Y.; Robotham, A.; Fernandes, S. M. *Glycobiology* **2015**, *25*, 767-773.
42. Gulati, S.; Schoenhofen, I. C.; Whitfield, D. M.; Cox, A. D.; Li, J.; St Michael, F. *PLoS Pathogens*, **2015**, *11*, e1005290.
43. Tsvetkov, Y. E.; Shashkov, A. S.; Knirel, Y. A.; Zahringer, U. *Carbohydr. Res.* **2001**, *331*, 233-237.
44. Nazarenko, E. L.; Shashkov, A. S.; Knirel, Y. A.; Ivanova, E. P.; Ovodov, Y. S. *Bioorg. Khim.* **1990**, *16*, 1426-1429.
45. Haseley, S. R.; Wilkinson, S. G. *Eur. J. Biochem.* **1997**, *250*, 617-623.
46. Hashii, N.; Isshiki, Y.; Iguchi, T., Hisatsune, K.; Kondo, S. *Carbohydr. Res.* **1996**, *287*, 225-245.
47. Hashii, N.; Isshiki, Y.; Iguchi, T.; Kondo, S. *Carbohydr. Res.* **2003**, *338*, 1063-1071.
48. Li, X.; Perepelov, A. V.; Wang, Q.; Senchenkova, S. N.; Liu, B., Shevelev, S. D.; Guo, X.; Shashkov, A. S.; Chen, W.; Wang, L.; Knirel, Y. A. *Carbohydr. Res.* **2010**, *345*, 1581-1587.
49. Knirel, Y. A.; Helbig, J. H.; Zahringer, U. *Carbohydr. Res.* **1996**, *283*, 129-139.
50. Edebrink, P.; Jansson, P. –E.; Bogwald, J.; Hoffman, J. *Carbohydr. Res.* **1996**, *287*, 225-245.
51. Twine, S. M.; Paul, C. J.; Vinogradov, E.; McNally, D. J.; Brisson, J. –R.; Mullen, J. A.; McMullin, D. R.; Jarrell, H. C.; Austin, J. W.; Kelly, J. F.; Logan, S. M. *FEBS J.* **2008**, *275*, 4428-4444.

52. Knirel, Y. A.; Vinogradov, E. V.; L'vov, V. L.; Kocharova, N. A.; Shashkov, A. S.; Dmitriev, B. A.; Kochetkov, N. K. *Carbohydr. Res.* **1984**, *133*, C5-C8.
53. Schaffer, C.; Messner, P. *FEMS Microbiol. Rev.* **2017**, *41*, 49-91.
54. Iwashkiw, J. A.; Voza, N. F.; Kinsella, R. L.; Feldman, M. F. *Mol. Microbiol.* **2013**, *89*, 14-28.
55. Nothaft, H.; Szymanski, C. M. *Nat. Rev. Microbiol.* **2010**, *8*, 765-778.
56. Abu-Qarn, M.; Eichler, J.; Sharon, N. *Curr. Opin. Struct. Biol.* **2008**, *18*, 544-550.
57. Knirel, Y. A.; Shevelev, S. D.; Perepelov, A. V. *Mendeleev Commun.* **2011**, *21*, 173-182.
58. Lewis, A. L.; Desa, N.; Hansen, E. E.; Knirel, Y. A.; Gordon, J. I.; Gagneux, P.; Nizet, V.; Varki, A. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 13552-13557.
59. Liu, F.; Aubry, A. J.; Schoenhofen, I. C.; Logan, S. M.; Tanner, M. E. *ChemBioChem* **2009**, *10*, 1317-1320.
60. Kaewsapsak, P.; Esonu, O.; Dube, D. H. *ChemBioChem* **2013**, *14*, 721-726.
61. Menard, R.; Schoenhofen, I. C.; Tao, L. M.; Aubry, A.; Bouchard, P.; Reid, C. W.; Lachance, P.; Twine, S. M.; Fulton, K. M.; Cui, Q.Z.; Hogues, H.; Purisima, E. O.; Sulea, T.; Logan, S. M. *Antimicrob. Agents Chem.* **2014**, *58*, 7430-7440.
62. Staaf, M.; Weintraub, A.; Widmalm, G. *Eur. J. Biochem.*, **1999**, *263*, 656-661.
63. Kondakova, A. N.; Perepelov, A. V.; Bartodziejska, B.; Shashkov, A. S.; Senchenkova, S. N.; Wykrota, M.; Knirel, Y. A.; Rozalski, A. *Carbohydr. Res.* **2001**, *333*, 241-249.
64. Thibault, P.; Logan, S. M.; Kelly, J. F.; Brisson, J. -R.; Ewing, C. P.; Trust, T. J.; Guerry, P. *J. Biol. Chem.* **2001**, *276*, 34862-34870.

65. Schirm, M.; Schoenhofen, I. C.; Logan, S. M.; Waldron, K. C.; Thibault, P. *Anal. Chem.* **2005**, *77*, 7774-7782.
66. Logan, S. M.; Kelly, J. F.; Thibault, P.; Ewing, C. P.; Guerry, P. *Mol. Microbiol.* **2002**, *46*, 587-597.
67. Schirm, M., Soo, E. C., Aubry, A. J., Austin, J., Thibault, P.; Logan, S. M. *Mol. Microbiol.* **2003**, *48*, 1579-1592.
68. Perepelov, A. V.; Shashkov, A. S.; Torgov, V. I.; Nazarenko, E. L.; Gorshkova, R. P.; Ivanova, E. P.; Gorshkovac, N. M.; Widmalma, G. *Carbohydr. Res.* **2005**, *340*, 69-74.
69. Le Quere, A. J. –L; Deakin, W. J.; Schmeisser, C.; Carlson, R. W.; Streit, W. R.; Broughton, W. J.; Forsberg, L. S. *J. Biol. Chem.* **2006**, *281*, 28981-28992.
70. Sharypova, L. A.; Chataigne, G.; Fraysse, N.; Becker, A.; Poinot, V. *Glycobiology* **2006**, *16*, 1181-1193.
71. Miller, M. G.; Forsberg, L. S.; Keating, D. H. *J. Bacteriol.* **2009**, *191*, 6988-7000.
72. Perepelov, A. V.; Shashkov, A. S.; Tomshich, S. V.; Komandrovab, N. A.; Nedashkovskaya, O. I. *Carbohydr. Res.* **2007**, *342*, 1378-1381.
73. Vinogradov, E.; Wilde, C.; Anderson, E. M.; Nakhamchik, A.; Lam, J. S.; Rowe-Magnus, D. A. *Carbohydr. Res.* **2009**, *344*, 484-490.
74. Vinogradov, E.; Frimmelova, M.; Toman, R. *Carbohydr. Res.* **2013**, *378*, 108-113.
75. Knirel, Y. A.; Vinogradov, E. V.; Shashkov, A. S.; Kochetkov, N. K.; L'vov, V. L.; Dmitriev, B. A. *Carbohydr. Res.* **1985**, *141*, C1-C3.
76. L'vov V. L.; Shashkov, A. S.; Dmitriev, B. A. *Bioorg. Khim.* **1987**, *13*, 223-233.

77. Knirel, Y. A.; Vinogradov, E. V.; Shashkov, A. S.; Dmitriev, B. A.; Kochetkov, N. K.; Stanislavsky, E. S.; Mashilova, G. M. *Eur. J. Biochem.* **1986**, *157*, 129-138.
78. Gil-Serrano, A. M.; Rodriguez-Carvajal, M. A.; Tejero-Mateo, P.; Espartero, J. L.; Menendez, M.; Corzo, J.; Ruiz-Sainz, J. E.; Buendia-Claveria, A. M. *Biochem. J.* **1999**, *342*, 527-535.
79. Shashkov, A. S.; Tul'skaya, E. M.; Streshinskaya, G. M.; Senchenkova, S. N.; Avtukh, A. N.; Evtushenko, L. I. *Carbohydr. Res.* **2009**, *344*, 2255-2262.
80. Tul'skaya, E. M.; Shashkov, A. S.; Streshinskaya, G. M.; Senchenkova, S. N.; Potekhina, N. V.; Kozlova, Y. I.; Evtushenko, L. I. *Biochemistry*, **2011**, *76*, 736-744.
81. Shashkov, A. S.; Streshinskaya, G. M.; Kozlova, Y. I.; Tul'skaya, E. M.; Senchenkova, S. N.; Arbatskii, N. P.; Bueva, O. V.; Evtushenko, L. I. *Biochemistry* **2012**, *77*, 511-517.
82. Knirel, Y. A.; Kocharova, N. A.; Shashkov, A. S.; Kochetkov, N. K. *Carbohydr. Res.* **1986**, *145*, C1-C4.
83. Knirel, Y. A.; Kocharova, N. A.; Shashkov, A. S.; Dmitriev, B. A.; Kochetkov, N. K.; Stanislavsky, E. S.; Mashilova, G. M. *Eur. J. Biochem.* **1987**, *163*, 639-652.
84. Muldoon, J.; Shashkov, A. S.; Senchenkova, S. N.; Tomshich, S. V.; Komandrova, N. A.; Romanenko, L. A.; Knirel, Y. A.; Savage, A. V. *Carbohydr. Res.* **2001**, *330*, 231-239.
85. Kenne, L.; Lindberg, B.; Schweda, E.; Gustafsson, B.; Holme, T. *Carbohydr. Res.* **1988**, *180*, 285-294.

86. Kandiba, L.; Aitio, O.; Helin, J.; Guan, Z.; Permi, P.; Bamford, D. H.; Eichler, J.; Roine, E. *Mol. Microbiol.* **2012**, *84*, 578-593.
87. Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E.; Gilbert, D.; Rice, L. B.; Scheld, M.; Spellberg, B.; Bartlett, J. *Clin. Infect. Dis.* **2009**, *48*, 1-12.
88. Ramphal, R.; Sadoff, J. C.; Pyle, M.; Silipigni, J. D. *Infect. Immun.* **1984**, *44*, 38-40.
89. Comer, J. E.; Marshall, M. A.; Blanch, V. J.; Deal, C. D.; Castric, P. *Infect. Immun.* **2002**, *70*, 2837-2845.
90. Castric, P. *Microbiology* **1995**, *141*, 1247-1254.
91. Smedley, J. G., III; Jewell, E.; Roguskie, J.; Horzempa, J.; Syboldt, A.; Stolz, D. B.; Castric, P. *Infect. Immun.* **2005**, *73*, 7922-7931.
92. Horzempa, J.; Held, T. K.; Cross, A. S.; Furst, D.; Qutyan, M.; Neely, A. N.; Castric, P. *Clin. Vaccine Immunol.* **2008**, *15*, 590-597.
93. Chen, X.; Varki, A. *ACS Chem Biol.* **2010**, *5*, 163-176.
94. Schoenhofen, I. C.; Vinogradov, E.; Whitfield, D. M.; Brisson, J. -R.; Logan, S. M. *Glycobiology*, **2009**, *19*, 715-725.
95. McNally, D. J.; Hui, J. P. M.; Aubry, A. J.; Mui, K. K. K.; Guerry, P.; Brisson, J. -R.; Logan, S. M.; Soo, E. C. *J. Biol. Chem.* **2006**, *281*, 18489-18498.
96. Schoenhofen I. C.; McNally, D. J.; Vinogradov, E.; Whifield, D.; Young, N. M.; Dick, S.; Wakarchuk, W. W.; Brisson, J. -R.; Logan, S. M. *J. Biol. Chem.* **2006**, *281*, 723-732.
97. Schoenhofen, I. C.; McNally, D. J.; Brisson, J. -R.; Logan, S. M. *Glycobiology* **2006**, *16*, 8C-14C.

98. McNally, D. J.; Schoenhofen, I. C.; Houliston, R. S.; Khieu, N. H.; Whitfield, D. M.; Logan, S. M.; Jarrell, H. C.; Brisson, J. –R. *ChemMedChem* **2008**, *3*, 55-59.
99. Guerry, P.; Ewing, C. P.; Schirm, M.; Lorenzo, M.; Kelly, J.; Pattarini, D.; Majam, G.; Thibault, P.; Logan, S. M. *Mol. Microbiol.* **2006**, *60*, 299-311.
100. Morrison, J. P.; Schoenhofen, I. C.; Tanner, M. E. *Bioorg. Chem.* **2008**, *36*, 312-320.
101. Schoenhofen, I. C.; Lunin, V. V.; Julien, J. –P; Li, Y.; Ajamian, E.; Matte, A.; Cygler, M.; Brisson, J. –R.; Aubry, A.; Logan, S. M.; Bhatia, S.; Wakarchuk, W. W.; Young, N. M. *J. Biol. Chem.* **2006**, *281*, 8907-8916.
102. Liu, F.; Tanner, M. E. *J. Biol. Chem.* **2006**, *281*, 20902-20909.
103. Rangarajan, E. S.; Proteau, A.; Cui, Q.; Logan, S. M.; Potetinova, Z.; Whifield, D.; Purisima, E. O.; Cygler, M.; Matte, A.; Sulea, T.; Schoenhofen, I. C. *J. Biol. Chem.* **2009**, *284*, 20989-21000.
104. Parker, J. L.; Day-Williams, M. J.; Tomas, J. M.; Stafford, G. P.; Shaw, J. G. *MicrobiologyOpen* **2012**, *1*, 149-160.
105. Cohen, M.; Varki, A. *OMICS: A Journal of Integrative Biology* **2010**, *14*, 455-464.
106. Hanashima, S. *Trends Glycosci. Glycotechnol.* **2011**, *23*, 111-121.
107. Komarova, B. S.; Ustyuzhanina, N. E.; Tsvetkov, Y. E.; Nifantiev, N. E. *Modern Synthetic Methods in Carbohydrate Chemistry*, Wiley – VCH Verlag GmbH & Co. KGaA **2013**, 125-159.
108. Haberman, J. M.; Gin, D. Y. *Org. Lett.* **2001**, *3*, 1665-1668.

109. Takahashi, T.; Tsukamoto, H.; Yamada, H. *Tetrahedron Lett.* **1997**, *38*, 8223-8226.
110. Boons, G. -J.; Demchenko, A. V. *Chem Rev.* 2000, *100*, 4539-4566.
111. De Meo, C.; Priyadarshani, U. *Carbohydr. Res.* **2008**, *343*, 1540-1542.
112. Demchenko, A. V.; Boons, G. J. *Tetrahedron Lett.* **1998**, *39*, 3065-3068.
113. Demchenko, A. V.; Boons, G. J. *Chem Eur. J.* **1999**, *5*, 1278-1283.
114. Schneider, R.; Freyhardt, C.; Schmidt, R. *Eur. J. Org. Chem.* **2001**, *2001*, 1655-1661.
115. Yu, C. -S.; Niikura, K.; Lin, C. -C.; Wong, C. -H. *Angew. Chem. Int. Ed.* **2001**, *40*, 2900-2903.
116. Gong, J.; Liu, H.; Nicholls, J. M.; Li, X. *Carbohydr. Res.* **2012**, *361*, 91-99.
117. Lu, K. -C.; Tseng, S. -Y.; Lin, C. -C. *Carbohydr. Res.* **2002**, *337*, 755-760.
118. Mandhapati, A. R.; Rajender, S.; Shaw, J.; Crich, D. *Angew. Chem. Int. Ed.* **2015**, *54*, 1275-1278.
119. De Meo, C.; Demchenko, A. V.; Boons, G. J. *J. Org. Chem.* **2001**, *66*, 5490-5497.
120. Meijer, A.; Ellervik, U. *J. Org. Chem.* **2004**, *69*, 6249-6256.
121. Tsvetkov, Y. E.; Nifantiev, N. E. *Synlett* **2005**, *9*, 1375-1380.
122. Ando, H.; Koike, Y.; Koizumi, S.; Ishida, H.; Kisa, M. *Angew. Chem. Int. Ed.* **2005**, *117*, 6917-6921.
123. Rich, J. R.; Wakarchuk, W. W.; Bundle, D. R. *Chem. Eur. J.* **2006**, *12*, 845-858.
124. Ando, H.; Koike, Y.; Ishida, H.; Kiso, M. *Tetrahedron Lett.* **2003**, *44*, 6883-6886.
125. Sherman, A. A.; Yudina, O. N.; Shashkov, A. S.; Menshov, V. M.; Nifant'ev, N. E. *Carbohydr. Res.* **2001**, *330*, 445-458.

126. Ikeda, K.; Miyomoto, K.; Sato, M. *Tetrahedron Lett.* **2007**, *48*, 7431-7435.
127. Sparks, M. A.; Williams, K. W.; Lukacs, C.; Schrell, A.; Priebe, G.; Spaltenstein, A.; Whitesides, G. M. *Tetrahedron.* **1993**, *49*, 1-12.
128. Adachi, M.; Tanaka, H.; Takahashi, T. *Synlett.* **2004**, *2004*, 609-614.
129. Tanaka, H.; Adachi, M.; Takahashi, T. *Chem. Eur. J.* **2005**, *11*, 849-862.
130. Crich, D.; Li, W. *J. Org. Chem.* **2007**, *72*, 2387-2391.
131. Crich, D.; Li, W. *J. Org. Chem.* **2007**, *72*, 7794-7797.
132. Tanaka, H.; Nishiura, Y.; Takahashi, T. *J. Am. Chem. Soc.* **2006**, *128*, 7124-7125.
133. Farris, M. D.; De Meo, C. *Tetrahedron Lett.* **2007**, *48*, 1225-1227.
134. Matthies, S.; Stallforth, P.; Seeberger, P. H. *J. Am. Chem. Soc.* **2015**, *137*, 2848-2851.
135. Hassan, M. I.; Lundgren, B. R.; Chauman, M.; Whifield, D. M.; Clark, B.; Schoenhofen, I. C.; Boddy, C. N. *Angew. Chem. Int. Ed.* **2016**, *55*, 12018-12021.
136. Glaze, P. A.; Watson, D. C.; Young, N. M.; Tanner, M. E. *Biochemistry* **2008**, *47*, 3272-3282.
137. Santra, A.; Xiao, A.; Yu, H.; Li, W.; Li, Y.; Ngo, L.; McArthur, J. B.; Chen, X.; *Angew. Chem.* **2018**, *130*, 2979-2983.
138. Tsvetkov, Y. E.; Shashkov, A. S.; Knirel, Y. A.; Zahringer, U. *Carbohydr. Res.* **2001**, *335*, 221-243.
139. Lee, Y. J.; Kubota, A.; Ishiwata, A.; Ito, Y. *Tetrahedron Lett.* **2011**, *52*, 418-421.
140. Williams, J. T.; Corcilius, L.; Kiefel, M. J.; Payne, R. J. *J. Org. Chem.* **2016**, *81*, 2607-2611.

141. Liu, H.; Zhang, Y.; Wei, R.; Andolina, G.; Li, X.; *J. Am. Chem. Soc.* **2017**, *139*, 13420-13428.
142. Chou, W. K.; Dick, S.; Wakarchuk, W. W.; Tanner, M. E. *J. Biol. Chem.* **2005**, *280*, 35922-35928.
143. Kancharla, P. K.; Crich, D. *J. Am. Chem. Soc.* **2013**, *135*, 18999-19007.
144. Dharuman, S.; Crich, D. *Chem. –Eur. J.* **2016**, *22*, 4535-4542.
145. Navuluri, C.; Crich, D. *Angew. Chem. Int. Ed.* **2013**, *52*, 11339-11342.
146. Hartmann, M.; Zbiral, E. *Monatsch. Chem.* **1989**, *120*, 899-906.
147. Rhind-Tutt, A. J.; Vernon, C. A. *J. Chem. Soc.* **1960**, 4637-4644.
148. Lemieux, R. U.; Hendricks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056-4062.
149. Jensen, H. H.; Bols, M. *Acc. Chem. Res.* **2006**, *39*, 259-265.
150. Smith, D. M.; Woerpel, K. A. *Org. Biomol. Chem.* **2006**, *4*, 1195-1201.
151. Bock, K.; Duus, J. O. *J. Carbohydr. Chem.* **1994**, *13*, 513-543.
152. Grindley, T. B. *In Glycoscience: Chemistry and Chemical Biology*; Fraser-Reid, B., Tatsuta, K., Thiem J., Eds.; Springer: Berlin, 2001, Vol. 1, pp 3-51.
153. Rao, V. S. R.; Qasba, P. K.; Balaji, P. V.; Chandrasekaran, R. *Conformation of Carbohydrates*; Harwood Academic Publishers: Amsterdam, 1998.
154. Tvaroska, L.; Taravel, F. R.; Utille, J. P.; Carver, J. P. *Carbohydr. Res.* **2002**, *337*, 353-367.
155. MacLean, L. L.; Vinogradov, E.; Pagotto, F.; Perry, M. B. *Carbohydr. Res.* **2011**, *346*, 2589-2594.

156. Filatov, A. V.; Wang, M.; Wang, W.; Perepelov, A. V.; Shashkov, A. S.; Wang, L.; Knirel, Y. A. *Carbohydr. Res.* **2014**, *392*, 21-24.
157. Vinogradov, E.; MacLean, L.; Xu, H. H.; Chen, W. *Carbohydr. Res.* **2014**, *390*, 42-45.
158. Mas Pons, J.; Dumont, A.; Sautejeau, G.; Fugier, E.; Baron, A.; Dukan, S.; Vauzeilles, B. *Angew. Chem. Int. Ed.* **2014**, *53*, 1275-1278.
159. Buda, S.; Crich, D. *J. Am. Chem. Soc.* **2016**, *138*, 1084-1092.
160. Furuhata, K. *Trends Glycosci. Glycobiol.* **2004**, *16*, 143-169.
161. Marra, A.; Sinaĳ, P. *Carbohydr. Res.* **1989**, *190*, 317-322.
162. Popik, O.; Dhakal, B.; Crich, D. *J. Org. Chem.* **2017**, *82*, 6142-6152.
163. de Meijere, A.; Kozhushkov, S. I.; Spaeth, T.; Zefirov, N. S. *J. Org. Chem.* **1993**, *50*, 502-505.
164. Chatgililoglu, C. *Acc. Chem. Res.* **1992**, *25*, 188-194.
165. Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797-3800.
166. Potter, G. T.; Jayson, G. C.; Miller, G. J.; Gardiner, J. M. *J. Org. Chem.* **2016**, *81*, 3443-3446.
167. Dess, P. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155-4156.
168. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849-3862.
169. Hutchins, R. O.; Hutchins, M. K. In *Comprehensive Organic Synthesis*; Torst, B. N., Fleming, I., Eds.; Pergamon Press; New York, 1991; Vol 8.
170. Price, N. P. J.; Furukawa, T.; Cheng, F.; Qi, J.; Chen, W.; Crich, D. *J. Antibiotics* **2014**, *67*, 405-414.

171. Luche, J.-L.; Rodriguez-Hahn, L.; Crabbe, P. *J. Chem. Soc., Chem. Commun.* **1978**, 601.
172. Czarniecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8273-8279.
173. Hori, H.; Nakajima, T.; Nishida, Y.; Ohru, H.; Meguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317-6320.
174. Haverkamp, J.; Spoomaker, T.; Dorland, L.; Vliegthart, J. F. G.; Schauer, R. *J. Am. Chem. Soc.* **1979**, *101*, 4851-4853.
175. Prytulla, S.; Lauterwein, J.; Klessinger, M.; Thiem, J. *Carbohydr. Res.* **1991**, *215*, 345-349.
176. Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165-195.
177. Rao, V. S. R.; Qasba, P. K.; Balaji, P. V.; Chandrasekaran, R. *Conformation of Carbohydrates*; Harwood Academic Publishers: Amsterdam, 1998.
178. Christian, R.; Schulz, G.; Brandstetter, H. H.; Zbiral, E. *Carbohydr. Res.* **1987**, *162*, 1-11.
179. Brown, E. B.; Brey, W. S., Jr.; Weltner, W., Jr. *Biochim. Biophys. Acta* **1975**, *399*, 124-130.
180. Flippen, J. L. *Acta Cryst.* **1973**, *B29*, 1881-1886.
181. Veluraja, K.; Rao, V. S. R. *Biochim. Biophys. Acta* **1980**, *630*, 442-446.
182. Sabesan, S.; Bock, K.; Lemieux, R. U. *Can. J. Chem.* **1984**, *62*, 1034-1045.
183. Bandgar, B. P.; Zbiral, E. *Monat für Chemie* **1991**, *122*, 1075-1088.
184. Dabrowski, U.; Friebolin, H.; Brossmer, R.; Supp, M., *Tetrahedron Lett.* **1979**, *20*, 4637-4640.

185. Haverkamp, J.; Van Halbeek, H.; Dorland, L.; Vliegthart, J. F. G.; Pfeil, R.;
186. Schauer, R. *Eur. J. Biochem.* **1982**, *122*, 305-311.
187. Paulsen, H.; Tietz, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 927-928.
188. Okamoto, K.; Kondo, T.; Goto, T., *Bull. Chem. Soc. Jpn.* **1987**, *60*, 637-643.
189. Hsu, C.-H.; Chu, K.-C.; Lin, Y.-S.; Han, J.-L.; Peng, Y.-S.; Ren, C.-T.; Wu, C.-Y.; Wong, C.-H. *Chem – Eur. J.* **2010**, *16*, 1754-1760.
190. Edward, J. T. *Chem. Ind.* **1955**, 1102-1104.
191. Foster, A. B.; Overend, W. G. *Chem. Ind.* **1955**, 566-567.
192. Overend, W. G.; Rees, C. W.; Sequira, J. S. *J. Chem. Soc.* **1962**, 3429-3440.
193. Bülow, A.; Meyer, T.; Olszewski, T. K., Bols, M. *Eur. J. Org. Chem.* **2004**, *2004*, 323-329.
194. Okada, Y.; Mukae, T.; Okajima, K.; Taira, M.; Fujita, M.; Yamada, H. *Org. Lett.* **2007**, *9*, 1573-1576.
195. Walvoort, M. T. C.; Dinkelaar, J.; van den Bos, L. J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *Carbohydr. Res.* **2010**, *345*, 1252-1263.
196. Heuckendorff, M.; Pedersen, C. M.; Bols, M. *Chem – Eur. J.* **2010**, *16*, 13982-13994.
197. Pedersen, C. M.; Marinescu, L. G.; Bols, M. *C. R. Chim.* **2011**, *14*, 17-43.
198. Schreiner, E.; Zbiral, E. *Liebigs Ann. Chem.* **1990**, *1990*, 581-586.
199. Dhakal, B.; Buda, S.; Crich, D. *J. Org. Chem.* **2016**, *81*, 10617-10630.
200. Howells, R. D.; McCown, J. D. *Chem. Rev.* **1977**, *77*, 69-92.
201. Stang, P. J.; Hanack, M.; Subramanian, L. R. *Synthesis* **1982**, *1982*, 85-126.
202. Dhakal, B.; Bohé, L.; Crich, D. *J. Org. Chem.* **2017**, *82*, 9263-9269.

203. Zefirov, N. S.; Koz'min, A. S.; Zhdankin, V. V.; Kirin, N. M.; Yur'eva, N. M.; Sorokin, V. D. *Chem. Script* **1983**, *22*, 195-200.
204. Zefirov, N. S.; Koz'min, A. S. *Acc. Chem. Res.* **1985**, *18*, 154-158.
205. Kiyooka, S.-i.; Kaneno, D.; Fujiyama, R. *Tetrahedron* **2013**, *69*, 4247-4258.
206. Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217-11223.
207. Mannerstadt, K.; Ekelöf, K.; Oscarson, S. *Carbohydr. Res.* **2007**, *342*, 631-637.
208. Kuznik, G.; Hörsch, B.; Kretzschmar, G.; Unverzagt, C. *Biorog. Med. Chem. Lett.* **1997**, *7*, 577-580.
209. Mukaiyama, T.; Mandai, H.; Jona, H. *Chem. Lett.* **2002**, *31*, 1182-1183.
210. Tamai, H.; Ando, H.; Tanaka, H.-N.; Hosoda-Yabe, R.; Yabe; Ishida, H.; Kiso, M. *Angew. Chem. Int. Ed.* **2011**, *50*, 2330-2333.
211. Stevens, C. L.; Glinski, R. P.; Taylor, K. G.; Blumbergs, P.; Sirokman, F. *J. Am. Chem. Soc.* **1966**, *88*, 2073-2074.
212. Hanessian, S. *Chem. Commun.* **1966**, 796-798.
213. Stevens, C. L.; Glinski, R. P.; Taylor, K. G.; Sirokman, F. *J. Org. Chem.* **1970**, *35*, 592-596.
214. Shirai, R.; Nakamura, M.; Hara, S.; Takayanagi, H.; Ogura, H.; *Tetrahedron Lett.* **1988**, *29*, 4449-4452.
215. Jensen, H. H.; Nordstrøm, L. U.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 9205-9213.
216. Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144-1253.
217. Crich, D. *J. Org. Chem.* **2011**, *76*, 9193-9209.

218. Adero, P. O.; Amarasekara, H.; Wen, P.; Bohé, L.; Crich, D. *Chem. Rev.* **2018**, *118*, 8242-8284.
219. Crich, D.; Li, W. *Org. Lett.* **2006**, *8*, 959-962.
220. Moumé-Pymbock, M.; Furukawa, T.; Mondal, S.; Crich, D. *J. Am. Chem. Soc.* **2013**, *135*, 14249-14255.
221. Dharuman, S.; Amarasekara, H.; Crich, D. *J. Org. Chem.* **2018**, *83*, 10334-10351.
222. Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169-173.
223. Martinelli, M. J.; Nayyar, N. K.; Moher, E. D.; Dhokte, U. P.; Pawlak, J. M.; Vaidyanathan, R. *Org. Lett.* **1999**, *1*, 447-450.
224. Martinelli, M. J.; Vaidyanathan, R.; Pawlak, J. M.; Nayyar, N. K.; Dhokte, U. P.; Doecke, C. W.; Zollars, L. M. H.; Moher, E. D.; Khau, V. V.; Kosmrlj, B. *J. Am. Chem. Soc.* **2002**, *124*, 3578-3585.
225. Lattrell, R.; Lohaus, G. *Liebigs Ann. Chem.* **1974**, 091-920.
226. Alber, R.; Dax, K.; Linz, R. W.; Stuetz, A. E. *Carbohydr. Res.* **1983**, *118*, C5-C6.
227. Dong, H.; Rahm, M.; Thot, N.; Deng, L.; Brinck, T.; Ramström, O. *Org. Biomol. Chem.* **2013**, *11*, 648-653.
228. Binkley, R. W.; Ambrose, M. G. J. *Carbohydr. Chem.* **1984**, *3*, 1-49.
229. Dhakal, B.; Crich, D., *J. Am. Chem. Soc.* **2018**, *140*, 15008-15015.
230. Rakotomanomana, N.; Lacombe, J.-M.; Pavice, A. *Carbohydr. Res.* **1990**, *197*, 318-323.

231. Qin, C.; Schumann, B.; Zou, X.; Pereira, C. L.; Tian, G.; Hu, J.; Seeberger, P. H.
J. Am. Chem. Soc. **2018**, **140**, 3120-3127.
232. Hassner A., Fibiger R., Andisik D. *J. Org. Chem.* **1984**, *49*, 4237-4244.

ABSTRACT**STEREOSELECTIVE SYNTHESIS OF LEGIONAMINIC ACID AND
PSEUDAMINIC ACID GLYCOSIDES**

by

Bibek Dhakal**May 2019****Advisor:** Dr. David Crich**Major:** Chemistry**Degree:** Doctor of Philosophy

Legionaminic acid (Leg) and pseudaminic acid (Pse) are deoxy acetamido analogues of the most common sialic acid, *N*-acetylneuraminic acid (NeuAc) that are found in microorganisms. Leg and Pse are rare but important class of sialic acids, as their glycosides are found in lipopolysaccharides and glycoproteins of several pathogenic Gram-negative bacteria including *Pseudomonas aeruginosa*, *Legionella pneumophila* serogroup 1, *Campylobacter jejuni*, and *Campylobacter coli*. As such, the stereoselective glycosylation of the equatorial Leg and Pse donors, and the role played by their side chain conformations in anomeric reactivity and selectivity are the focus of this thesis.

Chapter two describes the synthesis of a Leg donor in 15 straightforward steps and 17% overall yield from the commercially available *N*-acetylneuraminic acid. The synthesized legionaminic acid donor showed excellent equatorial selectivity when glycosylated with various primary, secondary and tertiary acceptors when using NIS/TfOH as activator at -78 °C in 1:2 acetonitrile/dichloromethane. The selectivity was, nevertheless, less than the 5-azido NeuAc donor synthesized in Chapter three. The 9-deoxy nature of the side chain made the donor less disarming and afforded greater

oxocarbenium-like character in the transition state, a major factor attributing to some loss in the glycosylation selectivity.

Chapter three describes the synthesis of an intermediate *5-epi*-NeuAc donor having the same C-5 configuration as pseudaminic acid. The glycosylation reactions of various primary and secondary alcohols including a sialic acid based acceptor with this donor under the standard glycosylation reaction conditions gave exquisite equatorial selectivity. Conformational analysis reveals the side chain of the donor to have undergone a conformational change from the predominant *gg*- to the less active *gt*-conformation in order to avoid the steric and dipolar repulsion between the C5-N5 bond and the side chain. This change in conformation offsets the negative effect of the axial azide at the 5-position. Additionally, the stereoselective synthesis of the NeuAc glycosides obtained from the 5-azido NeuAc donor was in apparent contradiction to the selectivity observed for the various literature 5-azido NeuAc donors, and showcased the importance of low temperature (-78 °C) in the control of glycosylation selectivity.

Chapter four describes the synthesis of a pseudaminic acid donor in 20 steps and 5% overall yield from the commercially available *N*-acetylneuraminic acid. Glycosylation reactions of various primary and hindered secondary alcohols under the standard glycosylation reaction conditions gave exquisite equatorial selectivity and good to excellent yields. The excellent selectivity observed is attributed to the inversion of configuration at the 5- and 7-positions, which creates a strong dipolar and steric repulsion between the C5-N5 bond and the side chain. To avoid this, the donor undergoes a conformational change from the predominant *gg*- to the least active *tg*-conformation and is rendered more selective in the glycosylation reaction. For selected glycosides,

regioselective reductions of the azides were performed to afford deprotected glycosides with differentially blocked amines, suitable for accessing the bacterial lipopolysaccharides.

Chapter five includes the overall conclusion of the dissertation, which is followed by the detailed experimental procedures employed in synthesizing all the sialic acid derivatives in chapter six.

AUTOBIOGRAPHICAL STATEMENTBIBEK DHAKAL

Education and Professional Experience

2013-Present	Ph.D. in organic chemistry at Wayne State University, Detroit under the supervision of Prof. David Crich
2011-2013	Lecturer for undergraduate students at National School of Sciences (NSS), Kathmandu, Nepal
2009-2011	M.Sc. in Organic Chemistry at Osmania University, Hyderabad, India

Publications

- **Dhakal, B.**; Crich, D. “Synthesis and Stereocontrolled Equatorially Selective Glycosylation Reactions of a Pseudaminic Acid Donor: Importance of the Side Chain Conformation, and Regioselective Reduction of Azide Protecting Groups” *J. Am. Chem. Soc.* **2018**, *140*, 15008-15015.
- **Dhakal, B.**; Bohé, L.; Crich, D. “Trifluoromethanesulfonate Anion as Nucleophile in Organic Chemistry” *J. Org. Chem.* **2017**, *82*, 9263-9269.
- Popik, O.*; **Dhakal, B.***; Crich, D. “Stereoselective Synthesis of the Equatorial Glycosides of Legionaminic Acid” *J. Org. Chem.* **2017**, *82*, 6142-6152 (* = contributed equally).
- **Dhakal, B.**; Buda, S.; Crich, D. “Stereoselective Synthesis of 5-epi- α -Sialosides Related to the Pseudaminic Acid Glycosides. Reassessment of Stereoselectivity of the 5-Azido-5-deacetamidodialyl Thioglycosides and Use of Triflate as Nucleophile in the Zbiral Deamination of Sialic Acids” *J. Org. Chem.* **2016**, *81*, 10617-10630. (Featured Article)

Conferences

- Presented an oral talk at **256th ACS National Meeting** at Boston, MA on August 21, 2018. Title: Synthesis of pseudaminic acid glycosides for potential applications in the treatment of bacterial infections.