Tools and reagents to study polysaccharide and glycolipid matabolism

Gold, K.H.

Reagents and Tools to Study

Polysaccharide and Glycolipid Metabolism

PROEFSCHRIFT

ter verkrijging van
de grad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden
volgens besluit van het College voor Promoties
te verdedigen op donderdag 19 mei 2011
klokke 16:15 uur

door

Karl Henrik Gold

Geboren te Örebro, Zweden in 1976

Promotiecommissie

Promotores:	Prof. Dr. G.A. van der Marel Prof. Dr. H.S. Overkleeft
Co-promotor:	Dr. J.D.C. Codée
Overige Leden:	Prof. Dr. J.M.F.G Aerts Prof. Dr. J. Brouwer Prof. Dr. R. Field Prof. Dr. J. Lugtenburg

"I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale."

Marie Curie

Table of Contents

List of Abbreviations

4MU	4-methylumbelliferone
Å	Angstrom
Ac	acetyl
AIBN	2,2'-azobis(isobutyronitrile)
Asn	asparagine
Asp	aspartic acid
BODIPY	boron-dipyrromethene
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
bs	broad singlet
BSP	1-benzensulfinyl piperidine
Bz	benzoyl
CDI	carbonyl diimidazole
COSY	correlation spectroscopy
CMP	cytidine monophosphate
CTH	ceramidetrihexose (a.k.a. Gb3)
δ	chemical shift
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
dm	doublet of multiplets
dt	doublet of triplets
dtd	doublet of triplets of doublets
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DCE	1,2-dichloroethane
DCI	4,5-dicyanoimidazole
DiBAL	diisobutylaluminum hydride
DIC	*N*,*N*'-diisopropylcarbodiimide
DiPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMP	2,2-dimethoxypropane
DPS	diphenylsulfoxide
ECM	extracellular matrix
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ELISA	enzyme-linked immunosorbent assay
ER	endoplasmic reticulum
ESI	electro spray ionization
Et	ethyl
eq	molar equivalents
FRET	fluorescence resonance energy transfer
Fuc	fucose
GAG	glucosamino glycan
Gal	galactose
GalNAc	*N*-acetyl galactosamine
Gb3	globotriaosylceramide (a.k.a. CTH)
GDP	guanosine diphosphate
GM1	monosialotetrahexosylganglioside
GLA	α-galactosidase A
Glc	glucose
GlcA	glucuronic acid
GlcNAc	*N*-acetyl glucosamine
GlcNAz	*N*-azidoacetyl glucosamine
Glu	glutamic acid
GPI	glycosylphosphatidylinositol
GSL	glycosphingolipid
GT	glycosyl transferase
h	hour(s)
HAS	hyaluronan synthase
His	histidine
HOBt	hydroxybenzotriazole
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
HWE	Horner-Wadsworth-Emmons
HYA	hyaluronic acid
Im	imidazole
^{i}Pr	isopropyl
IR	infrared
J	coupling constant
LDA	lithium diisopropylamide
Lev	levulinoyl
LG	leaving group
m	*multiplet*
Man	mannose
Me	methyl
min	minutes
Hz	hertz
MID	mass isotopomer distribution
m/z	mass-to-charge ratio

NBD	7-nitro-2-oxa-1,3-diazole
n-BuLi	*n*-butyllithium
NBS	*N*-bromosuccinimide
NDP	nucleoside diphosphate
Neu5Ac	*N*-acetylneuraminic acid
NIS	*N*-iodosuccinimide
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NP	nanoparticles
OCE	2-cyanoethoxy
PE	petroleum ether
Phth	phthalimido
PMB	para-methoxy benzyl
pTsOH	para-toluenesulfonic acid
q	quartet
RP	reversed phase
r.t.	room temperature
s	singlet
Sp	sphingosine
SPA	scintillation proximity assay
TBA	tetrabutylammonium
^{t}Bu	*tert*-butyl
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDPS	*tert*-butyl-diphenylsilyl
TBPP	tetrabenzylpyrophosphate
TBS	*tert*-butyl-dimethylsilyl
TCA	trichloroacetyl
td	triplet of doublets
tdt	triplet of doublets of triplets
TEMPO	2,2,6,6-tetramethylpiperidine 1-oxyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TfOH	trifluoromethansulfonic acid
Tf_2O	trifluoromethanesulfonic anhydride
THF	tetrahydrofuran
TLC	thin liquid chromatography
TMS	trimethylsilyl
TOCSY	total correlation spectroscopy
Troc	trichloroethyloxycarbonyl
TTBP	2,4,6-tris(*tert*-butyl)pyridine
Tyr	tyrosine
UDP	uridine diphosphate
UV/vis	ultraviolet/visible
Xyl	xylose

Chapter 1

General Introduction

The Biological Roles of Glycans and Their Conjugates

Carbohydrates and their derivatives, the glycans and glycoconjugates, are central in many biological processes. This diverse class of molecules is not only an important source of energy, but also plays a pivotal role in vital processes such as cell-cell recognition/adhesion, signal transduction, molecular trafficking, clearance, modification of physical properties and regulation of enzymatic activity. These functions are attributed to the complex and diverse structure of carbohydrates.[1]

The implication of protein glycosylation can roughly be divided into two parts, being the alteration of physiochemical or biological properties, although the two are often correlated. The physiochemical properties of proteins that are altered by glycosylation include: solubility, mass, viscosity and ionic charge. Glycosylation affects both the folding and stability of proteins, it provides structural integrity against proteolysis and mediates trafficking between cellular compartments.[2] Some of these

effects are exemplified by the glycosylation of α-galactosidase A, an enzyme encompassing *exo*-glycosidase activity, which is responsible for the hydrolysis of α-galactosyl linkages in various glycoconjugates. The glycosylation pattern of this enzyme is crucial for the formation of a soluble, active protein that will be targeted to its place of action, the lysosome.[3] Recently, the stabilizing effect of protein glycosylation, has been exploited in the development of pharmaceuticals with enhanced integrity.[4]

Glycosylation events may also affect enzyme activity and clearance. The activity or substrate recognition of an enzyme might be affected by glycosylation of the active site, forming a steric barrier.[5] Similarly, glycosylation of the substrate binding region may also alter the substrate-enzyme affinity. Glycosylation of a protein, distant from its active site can induce a conformational change of the enzyme and thus alter its activity.[6] The clearance of proteins from plasma is in some cases regulated by glycosylation of the protein and its sequent interaction with glycan receptor proteins on macrophages and liver endothelial cells.[7] One example is the Man/GalNAc-4-SO_4 receptor on hepatic endothelial cells, which recognizes sulfated GalNAc residues on the hormone lutropin and mediates its clearance from the circulation.[8]

The metabolism of glycan structures is regulated by glycosyltransferases, which synthesize new glycosidic linkages and glycosidases that cleave specific glycosidic linkages. These carbohydrate processing enzymes continuously modify glycan structures by specific recognition of their substrates and thus have a great impact on the regulation of cellular events.[9]

Considering the pivotal role of glycans and their conjugates, the metabolism of glycan structures is an important subject of investigation. A better understanding of glycan-modifying processes might shed light on their functions and could also give rise to possible entries for the development of novel pharmaceuticals aimed at glycan-specific pathological processes. The complex transformations facilitated by the glycoconjugate-processing enzymes described above, are generally difficult to monitor. The development of suitable probes for their monitoring represents an even greater challenge for the organic chemist. This introductory chapter will give an overview of a few selected glycoconjugate-processing enzymes and the various assays that have been developed for the monitoring of both their functions and derived products.

Glycosphingolipids – Function, Metabolism and Trafficking

Glycosphingolipids are a ubiquitous class of endogenous substances first described by the German physician Tudichum after isolation from brain extracts.[10] The glycosphingolipids consist of two parts; a lipid part that acts as a cell membrane anchor embedded in the lipid bilayer and a hydrophilic glycan part, which is protruding from the cell membrane. The glycan part of the glycosphingolipids encompasses great structural diversity and has a corresponding diverse set of functions. These functions include binding of microrganisms/toxins, cell-to-cell recognition/adhesion (mediated *via* glyan-protein or glycan-glycan interactions) and

initiation/modulation of signal transduction.[11] Glycosphingolipids have gained a lot of attention in recent years due to their involvement in many pathological conditions, such as cancer, microbial infections, diabetes, Alzheimer's disease and other neurological syndromes, as well as diseases of the cardiovascular and respiratory systems.[12]

The biosynthesis of glycosphingolipids involves *de novo* synthesis of ceramide on the membranes of the endoplasmic reticulum (ER), using L-serine and two equivalents of coenzyme A activated fatty acids. The ceramide is then glycosylated by either ceramide galactosyltransferase or glucosylceramide synthase, before further decoration of the glycan part by glycosyltransferases in the lumen of the Golgi apparatus. The glycosphingolipids are then transported to the cell-surface where they accumulate in glycosphingolipid- and cholesterol-enriched microdomains.[13] The catabolism of the glycosphingolipids is initiated by endocytosis of the glycosphingolipids at the cell membrane and their ensuing transport to the lysosomes. Glycosylsphingolipid-degrading enzymes (*i.e.* glycosidases and acid ceramidases) are located in these acidic compartments, which gradually strip the glycosphingolipids to regenerate the primary building blocks. A number of inheritable pathological disorders referred to as sphingolipidoses or lysosomal storage disorders, are characterized by a deficiency in the activity of specific glycosphingolipid degrading enzymes, located in the lysosome, which leads to the accumulation of the respective (glyco)sphingolipid substrates.[12,14]

The ability to monitor the metabolism and trafficking of specific glycosphingolipids is important for the investigation of the related pathological conditions. Both radiochemical and fluorescent methodologies have been applied in this context, which will be discussed below.

Radiochemical labeling of glycosphingolipids has been performed by catalytic addition of tritium to the unsaturated functionality of the sphingolipid part.[15] Although convenient in preparation, a disadvantage is that this procedure removes unsaturated centers, which results in an alteration of the physiochemical properties. Alternatively, two different methodologies for the synthesis of radio labeled cerebrosides with retained structural integrity have been developed. These include acylation of psychosine with a [1-$^{14}C_1$]-fatty acid[16] and enzymatic oxidation of the 6-OH of a galactocerebroside by galactose oxidase, followed by reduction with sodium [$^3H_4$]-borohydride.[17] The latter two methodologies produce probes that behave identically compared to the endogenous compounds in the biological system and they have been utilized for the investigation of galactocerebroside turnover in rats.[17] Synthesis of an isotopically-labeled (deuterium) ceramide trihexose (CTH) has also been performed and utilized as an internal standard for the quantitative determination of CTH levels in plasma of Fabry patients.[18]

A biotin-labeled GM1 ganglioside analogue (Figure 1, A) was used for the investigation of intracellular distribution and trafficking of the gangliosides, using immunolabeling with anti-biotin antibodies connected to gold particles for detection by electron microscopy.[19] Numerous fluorescent (glyco)sphingolipids (GSLs) have

been synthesized for the monitoring of GSL distribution and trafficking in living cells using fluorescence microscopy. A selection of fluorogenic glycosphingolipids that have been utilized for this purpose is depicted in Figure 1, B.

When performing studies with substrates labeled with fluorophores, it has to be kept in mind that the fluorogenic label may influence the distribution of the glycosphingolipid analogue within the lipid bilayer and its affinity for lipid rafts. A model study has showed that introduction of a short spacered fluorophore to the amine functionality of sphingosines do influence the distribution within the lipid bilayer and its participation in lipid rafts.[20] Introduction of fluorophores to the glycan part, on the other hand, may interfere with the biological effect of the glycosphingolipid (*i.e.* recognition of the glycan part by lectins *etc.*). Introduction of sterically demanding Lucifer yellow to the sialic acid of the GM1 ganglioside did not influence the recognition of cholera toxin, demonstrating that such modifications of the glycan part are possible with retained bioactivity of the glycosphingolipid.[21]

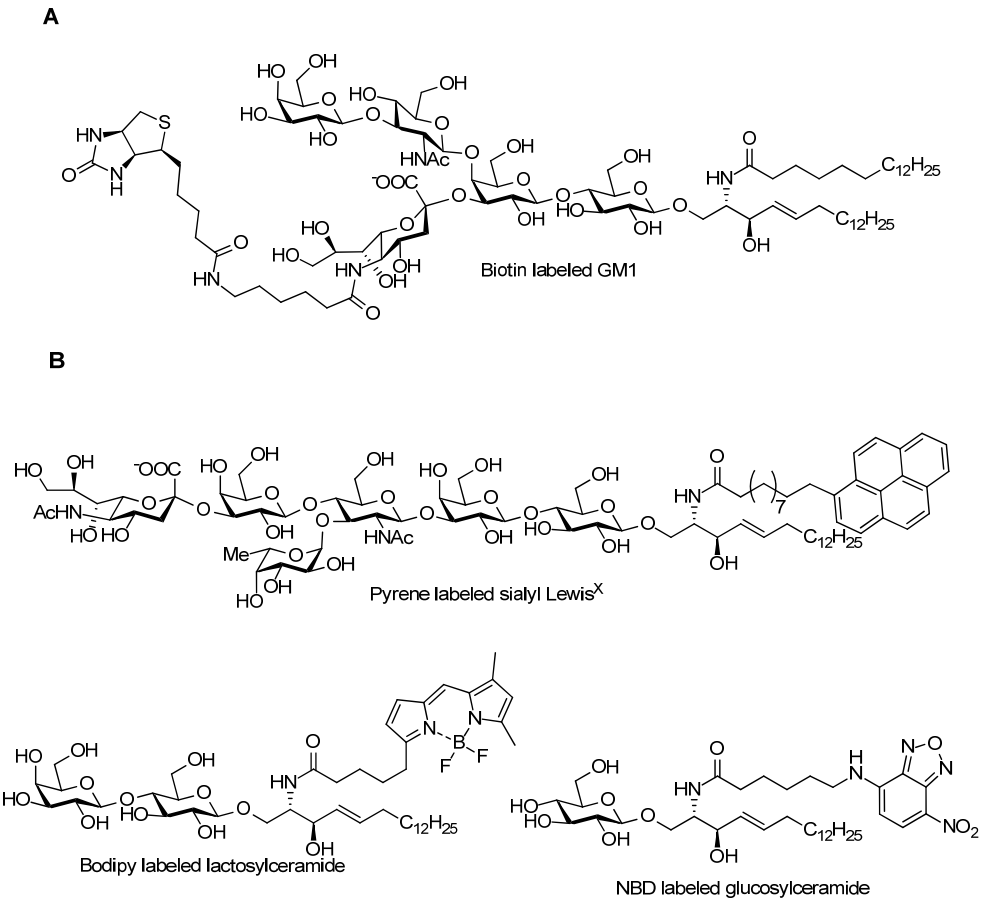


Figure 1. GSLs equipped with either a Biotin[19] or BODIPY,[22-25] NBD[25-28] and Pyrene[28,29] fluorophores.

As discussed in this section, the monitoring of glycosphingolipids is of importance for the understanding of various biological events. In **Chapter 2** of this thesis a new synthetic approach is presented for the preparation of globotriaosylsphingosine, a glycosphingolipid that is in the center of attention for investigations of the pathology of Fabry disease. This is followed by the development of a robust synthesis of a $^{13}C_5$-labeled D-*erythro*-sphingosine in **Chapter 3**. The labeled sphingosine was also decorated with a globotriose moiety for the formation of a $^{13}C_5$-labeled globotriaosylsphingosine, with the purpose of being used as an internal standard for the monitoring of globotriaosylsphingosine levels in plasma of Fabry patients.

Hyaluronidases – Monitoring of Glycosidase Activity

Hyaluronidases, enzymes capable of degrading hyaluronan, can be divided into three major groups. One of which is the prokaryote or bacterial hyaluronidases, also referred to as lyases or eliminases. These enzymes cleave the D-GlcNAc-(*β*-1,4)-D-GlcA glycosidic linkage, and introduce a double bond at the C4/C5-position of the glucuronic acid by an elimination reaction (Figure 2, A). The unsaturated product obtained by this catalytic mechanism enables a simple spectrophotometric assay for the enzyme activity, which is based on the monitoring of the formation of the *α*,*β*-unsaturated carboxylate product.[30]

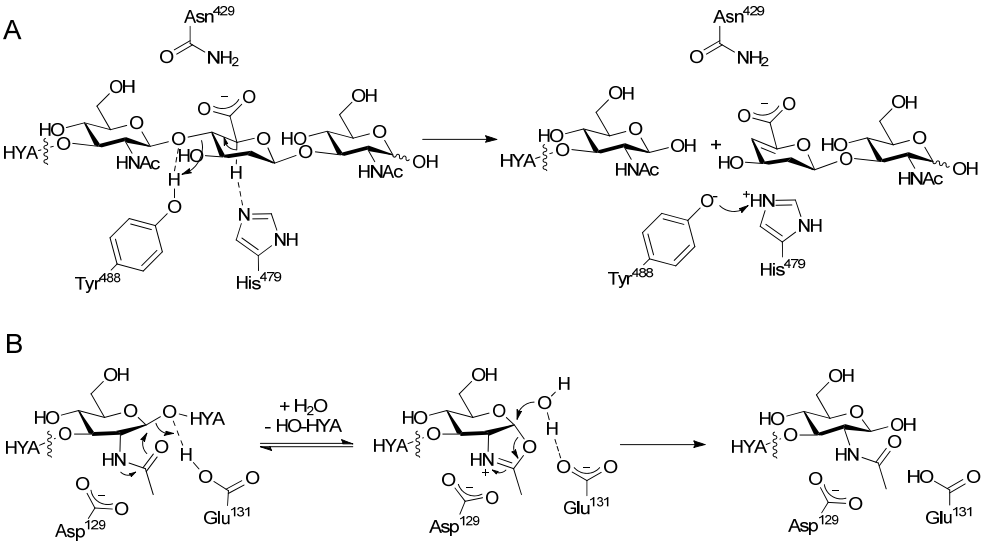


Figure 2. Catalytic residues involved in the enzymatic degradation of hyaluronate by bacterial (**A**) and vertebrate (**B**) hyaluronidases.

The other two types of hyaluronidases are present in eukaryotes and cleave one of the two glycosidic linkages present in hyaluronan. The *β*-endoglucuronidase is responsible for the cleavage of the D-GlcA-(*β*-1,3)-D-GlcNAc glycosidic linkage and is found in annelids, such as the leech *Herudo medicinalis*. Little is known about this enzyme, other than that it is a hydrolase possessing *endo*-*β*-glucuronidase activity.[31]

Significantly more is known about the last family of eukaryotic hyaluronidases. These are the *endo*-*β*-*N*-acetylhexosaminidases, which cleave the D-GlcNAc-(*β*-1,4)-D-GlcA glycosidic linkage by hydrolysis. This type of hyaluronidases predominately digests hyaluronic acid, although a lack of substrate specificity also enables degradation of chondroitin and chondroitin sulfate (albeit at a lower rate). The enzymatic hydrolysis of the glycosidic linkage is very different from the prokaryote mechanism and can best be explained as a substrate-assisted, double-displacement mechanism (Figure 2, B), which will be explained in further detail using the human hyaluronidase 1 (Hyal1) as an example.

The catalytic mechanism and essential catalytic amino acid residues for this enzyme have been elucidated by application of site-directed mutagenesis in combination with structural information.[32] The hyaluronidase encompasses a large, stretched-out cleft containing mostly hydrophobic and positively-charged amino acid residues that ensures a strong binding of the negatively-charged hyaluronate. Upon binding to the enzyme, the carbonyl of the (-1) GlcNAc residue is aligned in a favorable position for nucleophilic attack at the anomeric carbon of the same (-1) residue (Figure 2, B). The alignment of the amide functionality results in a deviation of conformation from a normal chair to a distorted boat, which positions the glycosidic oxygen in the proximity of the catalytic glutamic acid residue (Glu^{131}). Protonation of the leaving group (glycosidic oxygen) is followed by attack of the GlcNAc carbonyl oxygen at the anomeric position. Concomitant departure of the leaving group forms a positively-charged oxazolinium intermediate with inversion of configuration at the anomeric center, which is stabilized by a negatively-charged aspartate residue (Asp^{129}). Activation of water by the negatively-charged glutamate (Glu^{131}) with ensuing attack at the anomeric carbon leads to hydrolysis of the intermediate oxazolinium, with a second inversion at the anomeric position. This step also re-protonates the glutamic acid for the next catalytic cycle. In addition to hydrolytic activity, hyaluronidases also possess transglycosidase activity. The only known acceptors for this activity are other hyaluronan fragments (*i.e.* forming extended hyaluronan fragments).

All the genes in the human genome that code for hyaluronidases (HYAL1–4, PH20/SPAM1 and the pseudogene PHYAL1) do not only show great sequence homology, but there is also a significant homology of the hyaluronidase enzymes between different species. Two of the mammalian hyaluronidases (Hyal1 and Hyal2) are largely responsible for the degradation of hyaluronan in somatic tissues. Hyal1 is a lysosomal hyaluronidase that was the first of the somatic hyaluronidases to be isolated and characterized. It can utilize any size of hyaluronan as substrate and produces HYA-tetrasaccharides as the final hydrolytic products. Hyal2 is anchored to the plasma membrane by a glycosyl-phosphatidylinositol (GPI) link, but can also exist in a soluble form, detached from the linker. Hyal2 degrades high-molecular weight hyaluronan into ~20 kDa products, which are then further processed by Hyal1 in the lysosome. The smaller hyaluronic acid fragments are then degraded into

monosaccharide units by two lysosomal *exo*-glycosidases, namely *exo*-*β*-glucuronidase and *exo*-*β*-*N*-acetylglucosaminidase.

Following the initial synthesis of macromolecular hyaluronan by the various hyaluronan synthases located at the cell membrane, the catabolic hyaluronidases produce a wide distribution of hyaluronan fragments of varying length. The great size distribution of hyaluronan fragments produces a wide range of (and even opposing) biological effects.[33] The involvement of hyaluronan in a number of pathological conditions and the activities of the hyaluronidases that produce the corresponding HYA-fragments are of great interest and subject of numerous investigations.[33,34]

A great aid in these investigations would be the availability of hyaluronidase probes that can be used for continuous monitoring of the enzyme activity in order to deduce their involvement in various pathological processes. Physiochemical, radiochemical, spectrometric and ELISA-like assays have all been developed to monitor hyaluronidase activity for this purpose.

The early hyaluronidase assays took advantage of the measurable changes in physiochemical properties, such as viscosity[35] or turbidity.[36] In these assays, hydrolysis of the glycosidic linkages in hyaluronan results in decreasing viscosity or turbidity. The viscosity assay is based on the difference in viscosity between high molecular weight hyaluronan, which has a higher viscosity as compared to smaller hyaluronan fragments. The turbidity assay is based on the clotting capacity of high molecular weight hyaluronan with acidified serum albumin, a property which is not shared by low molecular weight hyaluronan fragments.

In order to set up a radiochemical assay, hyaluronan was initially *N*-deacetylated with hydrazine and then *N*-acetylated with [^{3}H]-acetic anhydride.[37] The labeled hyaluronan was then subjected to the action of hyaluronidases, followed by scintillation quantification after removal of the non-hydrolysed hyaluronan by precipitation with cetylpyridinium chloride.

The first ELISA-based assay utilized hyaluronan-coated microtiter plates that were subjected to hyaluronidase degradation. The remaining hyaluronan was then absorbed by hyaluronectin - a hyaluronic acid-binding proteoglycan,[38] which subsequently was reacted with anti-hyaluronectin antibodies, conjugated with an alkaline-phosphatase. The hyaluronidase activity, which is proportional to the activity of the phosphatase enzyme, was then monitored by spectroscopy after addition of *p*-nitrophenyl phosphate.[39] In a similar assay, hyaluronan was first partially functionalized with biotin before being covalently attached to a microtiter plate. The hyaluronidase activity was then monitored at the end of the enzymatic reaction by a quenching and washing procedure, followed by quantification of the residual biotin-labeled substrate by application of an avidine-peroxidase reaction visualized by means of spectroscopy.[40]

Hydrolysis of glycosidic linkages by hyaluronidases creates an increased amount of reducing-end sugars. Transformation of the reducing-ends into chromogenic and/or fluorogenic substrates have been applied for the determination of hyaluronidase activity. A combined HPLC purification/identification protocol of hyaluronan, with

post-column derivatization using 2-cyanoacetamide was developed[41] and utilized for kinetic and mechanistic studies of the bovine testicular hyaluronidase.[42] The Morgan-Elson method for determination of *N*-acetylglucosamine residues using *p*-dimethylaminobenzaldehyde under acidic conditions (Ehrlich's reagent),[43] has been applied for both colorimetric and fluorometric determination of hyaluronidase activity in relation to various diseases.[44,45]

Hyaluronidase assays that are based on a change in absorbance of chemical dyes and gold nanoparticles (AuNP's) depending on the local microenvironment have also been developed. For instance, the maximum absorbance wavelength of a carbocyanine dye is shifted, when bound to macromolecular hyaluronan. The absorbance reaches normal values upon degradation to smaller hyaluronan fragments, which do not have the same affinity for the dye.[46] A similar change in absorption spectra is also utilised in a colorimetric assay based on the interaction of positively charged AuNP's and hyaluronan. Macromolecular hyaluronan forms, in contrast to smaller hyaluronan fragments, clusters with AuNP's, which is at the basis of the change in specific absorbance of the AuNP's.[47]

A Fluorescence Resonance Energy Transfer (FRET) system using hyaluronan which has been exposed to an arbitrary, partial labeling of the carboxylic acids with both fluorescein amine and rhodamine B amine, has been developed for the continuous monitoring of hyaluronidases.[48]

The synthesis of suitable probes for the monitoring of hyaluronidase activity represents a big challenge for organic chemists. **Chapter 4** and **5** of this thesis describe the synthesis of hyaluronic acid dimers and tetramers bearing a fluorogenic leaving group as potential probes for a direct and continuous monitoring of hyaluronidase activity.

Glycosyltransferases – Probing Activity and Function

As outlined above, degradation of glycoconjugates is an important biological process for the metabolism of glycoconjugates. Dysfunctional enzymes involved in these processes may result in pathological conditions for the organism in question. Of equal importance is the biosynthesis of glycoconjugates, a process mainly performed by glycosyltransferases (GTs).[49] The GTs utilize activated carbohydrate donors in various forms for the glycosylation of a wide variety of acceptors such as proteins, other carbohydrates, fatty lipids and foreign organic molecules (xenobiotics). The latter is exemplified by glucuronidation and excretion of the secondary metabolites *via* the bile or urine and represent an important route for the clearance of potentially toxic organic molecules from our system.[50] The most common form of activated carbohydrate donors are sugar mono- or diphosphonucleotides (*e.g.*, CMP-Neu5Ac, UDP-GlcNAc, GDP-Fuc, *etc.*), which generally are referred to as Leloir donors.[9] Other forms of activated carbohydrates also exist such as unsubstituted phosphates (sugar-1-phosphates) and lipid phosphates (dolichol (pyro)phosphates)[51] that are employed as donors by non-Leloir enzymes. Examples of the various activated donors are represented in Figure 3.

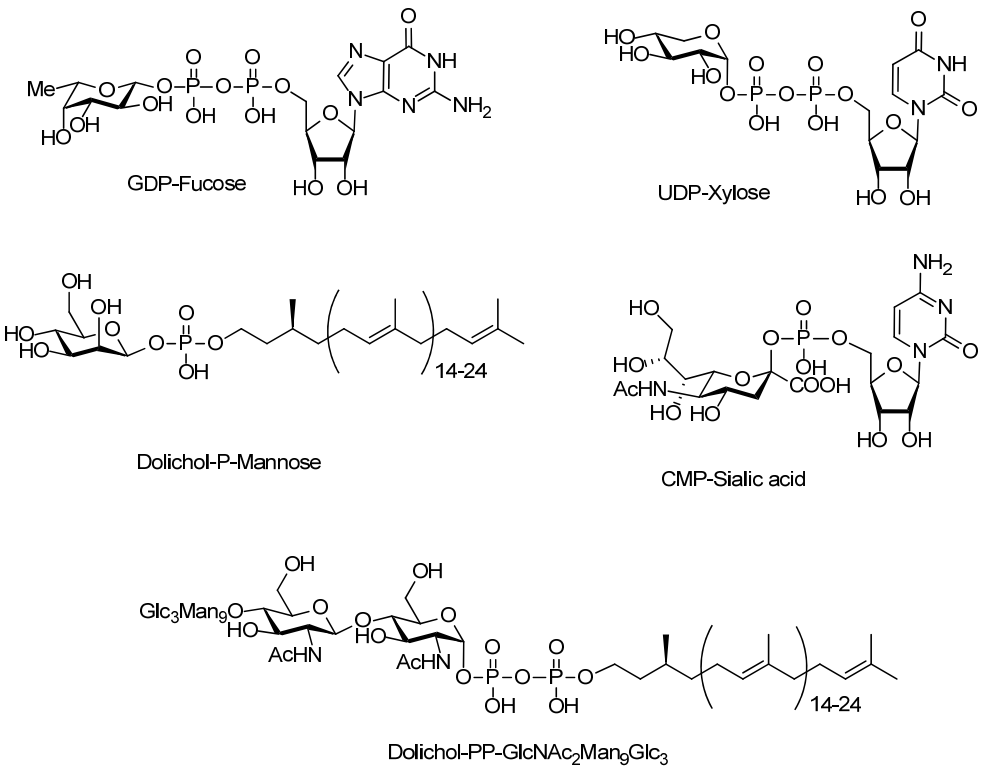


Figure 3. Examples of activated carbohydrate donors utilized by glycosyltransferases.

The GTs represent 1–2% of the total gene products of an organism and the number of identified GTs in man exceeds two hundred.[52] The GTs are generally selective when it comes to substrate (donor/acceptor) specificity and product outcome (one enzyme – one linkage paradigm), although exceptions to this rule have been observed.

The glycosyltransferases follow a generic mechanism in which the activated carbohydrate is transferred to an acceptor with a concomitant loss of the (pyro)phosphate leaving group. The glycosylation reaction can produce two different stereo chemical outcomes in the formation of the new glycosidic linkage, by either retaining or inversion of the anomeric configuration of the glycosyl donor, depending on the mechanism (Figure 4).[52]

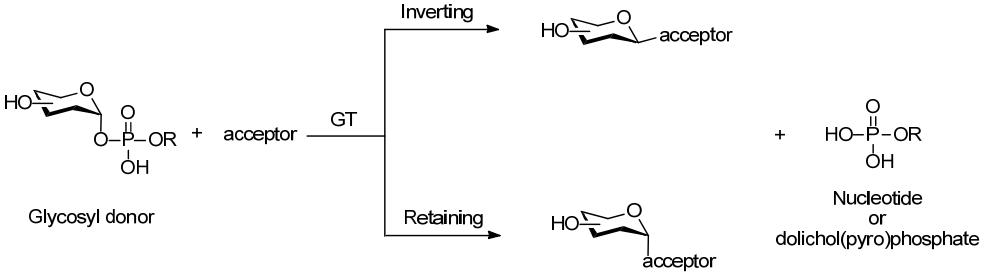


Figure 4. Retaining and inverting glycosyltransferase reactions.

Most of the GTs are located in the ER and Golgi apparatus, where they are organized in assembly lines for the construction of a variety of complex protein and lipid associated glycans.[53] Many of these GTs are involved in sequential glycosylation reactions, where the product of one glycosylation becomes the substrate for the next glycosylation.

The importance of glycosylation reactions and the implication of the corresponding products for essential processes in organisms, make GTs important targets for drug discovery.[54] To date there are only two glycosyltransferase inhibitors used in clinic, namely ethambutol and miglustat. Ethambutol inhibits arabinosyltransferase, which is involved in the construction of the mycobacterial cell wall and is one of five drugs that constitute a multi-drug regimen against *Mycobacterium tuberculosis*.[55] Miglustat is an orphan drug for the treatment of Gaucher patients. Gaucher disease is characterized by the accumulation of glucosylceramide in the lysosomes due to a deficiency of the glucocerebrosidase (the glycosidase responsible for the degradation of glucosylceramide to glucose and ceramide). Miglustat is an inhibitor of the glycosylceramide synthase, which results in diminished production and thus the abundance of glycosphingolipids in the system.[56] Monitoring of the various activities and functions of GTs constitute an important source of information, which can be utilized for the development of novel pharmaceutical treatments.

The GTs can be assayed in numerous ways, depending on the type of information required. For instance, the activity of GTs can be assayed by monitoring of the turnover of either one of the reactants or products involved in the generic glycosyltransferase reaction (Figure 4). The ideal GT activity assay is performed using continuous monitoring and saturating conditions of the substrates. Unfortunately, GT reactions generate no measurable change in UV/vis absorbance and are therefore difficult to monitor in a continuous (real time) fashion. Additionally, the GTs that are present in natural sources are often encountered in low concentrations, which therefore requires sensitive methods for the monitoring of the reactions. To overcome these problems various chromatographic, immunological and radiochemical methods have been developed which encompass enhanced sensitivity for the monitoring of glycosyltransferase activity.

Radiochemical methods are popular due to their sensitivity and the accessibility of commercially available radio-labeled sugar nucleotides. The general experiment includes an acceptor, a glycosyltransferase and the radio labeled sugar nucleotide. Aliquots of the reaction mixture can be removed at different time-intervals for purification of the product and determination of the reaction progression.[57] These experiments require that the non-reacted sugar nucleotides are separated from the glycosylated product prior to detection by scintillation quantification. Continuous monitoring of glycosyltransferases is possible by a scintillation proximity assay (SPA). The acceptors in these experiments are first attached to an SPA bead, which only emits light when in close contact to a radiation-emitting source (*i.e.* when the acceptor is glycosylated with a radiolabeled sugar nucleotide). In a high throughput screening

campaign for the hit identification of fucosyltransferase inhibitors, close to 800,000 potential inhibitors were screened utilizing SPA.[58]

Immunological assays have the advantage that the reaction products can be identified. These types of experiments are usually set up by the immobilization of acceptors to a microtiter plate, which then are subjected to the action of a glycosyltransferase and the corresponding sugar nucleotides. Product specific antibodies or lectins are then bound to the immobilized products that are further reacted with enzyme-linked anti-antibodies. The activity of the conjugated enzymes is proportional to the amount of glycosylated product and can thus be quantified.[59]

A direct approach for the monitoring of glycosyltransferase activity is presented by the Transscreener™ methodology in which the formation of the nucleotide is measured. The progression of the reaction is monitored by application of nucleoside diphosphate-specific antibodies, pre-bound to fluorescent nucleotides. The glycosyltransferase reaction liberates NDP's, which then displace the fluorescent tracer that is bound to the antibody resulting in a change of fluorescence intensity. This type of assay has successfully been applied for a row of different enzyme classes (*i.e.* glycosyltransferases, kinases and sulfotransferases) involved in a variety of cellular regulation processes.[60]

Chromatographic and fluorescence-based glycosyltransferase assays have become increasingly popular, due to their sensitivity and ease of application. The glycosyltransferase reaction can be monitored by application of fluorescently labeled donor substrates[61] or acceptors[62]. The former methodology was applied for the investigation of sialyltransferase activity using a CMP-9-fluoresceinyl-Neu5Ac as donor, which was used in place of the routinely used radio labeled CMP-Neu5Ac.[61] The fluorescent substrate-bound acceptor was quantified after gel filtration showing an up to 1000-fold increased sensitivity, compared to the radio labeled methodology. An assay to monitor the activity of glucosylceramide synthase and lactosylceramide synthase has been developed using fluorescent acceptor species. NBD-labeled ceramide and glucosylceramide were successfully utilized for the determination of glucosylceramide synthase and lactosylceramide synthase activities respectively in cell lysates by normal-phase HPLC detection.[62]

Fluorescence resonance energy transfer (FRET) has also been applied in glycosyltransferase activity assays.[63,64] These methodologies involve the synthesis of fluorescently labeled acceptors and sugar nucleotide donors that must be accepted by the glycosyl transferase to be monitored. Successful glycosylation of the acceptors leads to a FRET signal, which enables a continuous monitoring of the enzyme activity.

Fluorescently labeled sugar nucleotides can also be applied in ligand-displacement experiments by the monitoring of fluorescence anisotropy. The basis for these assays is that the fluorescence polarization[65] of a molecule is dependent on its surrounding (*i.e.* bound to a protein or free in solution), due to the difference in tumbling rates between the free and bound fluorophore. In this fashion fluorescent sugar nucleotides, labeled at either the sugar[66] or nucleoside[67], can be utilized for the investigation of potential glycosyltransferase inhibitors.

Glycosyltransferases can also be used for the identification of various glycoconjugates. For instance, azide-modified *N*-acetyl glucosamine was utilized for the incorporation of an azide-functionality in GlcNAc modified proteins in cells. The azide was then applicable for functionalisation *via* the Staudinger-Bertozzi reaction, giving suitably labeled glycoproteins that not only could be identified, but it was also concluded at what position glycosylation took place on the protein.[68]

These techniques show that the function of specific glycosyltransferases and their glycosylated products can be elucidated which may result in the development of novel pharmaceutical treatments. In **Chapter 6** a novel synthetic method is presented for the synthesis of pyrophosphates utilizing the powerful phosphorylating property of phosphoramidites. The presented methodology has been applied in the synthesis of a number of (non)-modified sugar nucleotides.

Conclusion

This chapter provides a summary of the assays that have been developed for selected examples of glycosidases and glycosyltransferases. The research presented in this thesis aims at the development of novel chemical methodologies or application of known chemical transformations for the synthesis of probes, which are amenable for the monitoring of enzymes involved in carbohydrate metabolism.

References and footnotes

1. Varki, A. *Glycobiology* **1993,** *3* (2), 97-130.
2. Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993,** *218* (1), 1-27.
3. Ioannou, Y. A.; Zeidner, K. M.; Grace, M. E.; Desnick, R. J. *Biochem. J.* **1998,** *332*, 789-797.
4. Sola, R. J.; Griebenow, K. *J. Pharm. Sci.* **2009,** *98* (4), 1223-1245.
5. Wyss, D. F.; Wagner, G. *Curr. Opin. Biotechnol.* **1996,** *7* (4), 409-416.
6. Rasmussen, J. R. *Curr. Opin. Struct. Biol.* **1992,** *2* (5), 682-686.
7. Drickamer, K. *Cell* **1991,** *67* (6), 1029-1032.
8. Roseman, D. S.; Baenziger, J. U. *Proc. Natl. Acad. Sci. U. S. A.* **2000,** *97* (18), 9949-9954.
9. Ohtsubo, K.; Marth, J. D. *Cell* **2006,** *126* (5), 855-867.
10. Tudichum, J. L. W., *The Chemical Constitution of the Brain*. Bailliére, Tindall & Cox, London: **1884**.
11. Hakomori, S. *Glycoconjugate J.* **2000,** *17* (3-4), 143-151.
12. Wennekes, T.; van den Berg, R.; Boot, R. G.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. *Angew. Chem., Int. Ed.* **2009,** *48* (47), 8848-8869.
13. Simons, K.; Ikonen, E. *Nature* **1997,** *387* (6633), 569-572.
14. Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999,** *38* (11), 1532-1568.
15. Di Cesare, J. L.; Rapport, M. M. *Chem. Phys. Lipids* **1974,** *13* (4), 447-452.
16. Kopaczyk, K. C.; Radin, N. S. *J. Lipid Res.* **1965,** *6* (1), 140-145.
17. Hajra, A. K.; Bowen, D. M.; Kishimoto, Y.; Radin, N. S. *J. Lipid Res.* **1966,** *7* (3), 379-386.
18. Mills, K.; Johnson, A.; Winchester, B. *FEBS Lett.* **2002,** *515* (1-3), 171-176.
19. Mobius, W.; Herzog, V.; Sandhoff, K.; Schwarzmann, G. *J. Histochem. Cytochem.* **1999,** *47* (8), 1005-1014.
20. Wang, T.-Y.; Silvius, J. R. *Biophys. J.* **2000,** *79* (3), 1478-1489.
21. Spiegel, S. *Biochemistry* **1985,** *24* (21), 5947-5952.

22. Mikhalyov, II; Molotkovsky, J. G. *Rus. J. Bioorg. Chem.* **2003,** *29* (2), 168-174.
23. Singh, R. D.; Puri, V.; Valiyaveettil, J. T.; Marks, D. L.; Bittman, R.; Pagano, R. E. *Mol. Biol. Cell* **2003,** *14* (8), 3254-3265.
24. Liu, Y. D.; Bittman, R. *Chem. Phys. Lipids* **2006,** *142* (1-2), 58-69.
25. Peters, C.; Billich, A.; Ghobrial, M.; Hogenauer, K.; Ullrich, T.; Nussbaumer, P. *J. Org. Chem.* **2007,** *72* (5), 1842-1845.
26. Schwarzmann, G.; Wendeler, M.; Sandhoff, K. *Glycobiology* **2005,** *15* (12), 1302-1311.
27. Babia, T.; Kok, J. W.; Hulstaert, C.; Deweerd, H.; Hoekstra, D. *Int. J. Cancer* **1993,** *54* (5), 839-845.
28. Gege, C.; Oscarson, S.; Schmidt, R. R. *Tetrahedron Lett.* **2001,** *42* (3), 377-380.
29. Ettmayer, P.; Billich, A.; Baumruker, T.; Mechtcheriakova, D.; Schmid, H.; Nussbaumer, P. *Bioorg. Med. Chem. Lett.* **2004,** *14* (6), 1555-1558.
30. Shimada, E.; Matsumura, G. *J. Biochem. (Tokyo, Jpn.)* **1980,** *88* (4), 1015-1023.
31. Linker, A.; Meyer, K.; Hoffman, P. *J. Biol. Chem.* **1960,** *235* (4), 924-927.
32. Zhang, L.; Bharadwaj, A. G.; Casper, A.; Barkley, J.; Barycki, J. J.; Simpson, M. A. *J. Biol. Chem.* **2009,** *284* (14), 9433-9442.
33. Stern, R.; Asari, A. A.; Sugahara, K. N. *Eur. J. Cell Biol.* **2006,** *85* (8), 699-715.
34. Lebel, L. *Adv. Drug Delivery Rev. 7* (2), 221-235.
35. Swyer, G. I. M.; Emmens, C. W. *Biochem. J.* **1947,** *41* (1), 29-34.
36. Dorfman, A.; Ott, M. L. *J. Biol. Chem.* **1948,** *172* (2), 367-375.
37. Coulson, C. J.; Girkin, R. *Anal. Biochem.* **1975,** *65* (1-2), 427-434.
38. Girard, N.; Courel, N. M.; Delpech, B. *Eur. J. Canc. Clin. Oncol.* **1987,** *23* (11), 1753-1753.
39. Delpech, B.; Bertrand, P.; Chauzy, C. *J. Immunol. Methods* **1987,** *104* (1-2), 223-229.
40. Frost, G. I.; Stern, R. *Anal. Biochem.* **1997,** *251* (2), 263-269.
41. Cramer, J. A.; Bailey, L. C. *Anal. Biochem.* **1991,** *196* (1), 183-191.
42. Cramer, J. A.; Bailey, L. C.; Bailey, C. A.; Miller, R. T. *Biochim. Biophys. Acta* **1994,** *1200* (3), 315-321.
43. Morgan, W. T. J.; Elson, L. A. *Biochem. J.* **1934,** *28* (3), 988-995.
44. Muckenschnabel, I.; Bernhardt, C.; Spruss, T.; Dietl, B.; Buschauer, A. *Cancer Letters* **1998,** *131* (1), 13-20.
45. Kucur, M.; Karadag, B.; Isman, F. K.; Ataev, Y.; Duman, D.; Karadag, N.; Ongen, Z.; Vural, V. A. *Bratisl. Med. J.* **2009,** *110* (1), 21-26.
46. Benchetrit, L. C.; Pahuja, S. L.; Gray, E. D.; Edstrom, R. D. *Anal. Biochem.* **1977,** *79* (1-2), 431-437.
47. Kim, J. W.; Kim, J. H.; Chung, S. J.; Chung, B. H. *Analyst* **2009,** *134* (7), 1291-1293.
48. Zhang, L. S.; Mummert, M. E. *Anal. Biochem.* **2008,** *379* (1), 80-85.
49. Watkins, W. M. *Carbohydr. Res.* **1986,** *149* (1), 1-12.
50. Ritter, J. K. *Chem.-Biol. Interact.* **2000,** *129* (1-2), 171-193.
51. Imperiali, B.; Hendrickson, T. L. *Bioorg. Med. Chem.* **1995,** *3* (12), 1565-1578.
52. Lairson, L. L.; Henrissat, B.; Davies, G. J.; Withers, S. G. *Annu. Rev. Biochem.* **2008,** *77*, 521-555.
53. Czlapinski, J. L.; Bertozzi, C. R. *Curr. Opin. Chem. Biol.* **2006,** *10* (6), 645-651.
54. Brown, J. R.; Crawford, B. E.; Esko, J. D. *Crit. Rev. Biochem. Mol. Biol.* **2007,** *42* (6), 481-515.
55. Barry, C. E. *Biochem. Pharmacol.* **1997,** *54* (11), 1165-1172.
56. Cox, T. M.; Aerts, J.; Andria, G.; Beck, M.; Belmatoug, N.; Bembi, B.; Chertkoff, R.; Vom Dahl, S.; Elstein, D.; Erikson, A.; Giralt, M.; Heitner, R.; Hollak, C.; Hrebicek, M.; Lewis, S.; Mehta, A.; Pastores, G. M.; Rolfs, A.; Miranda, M. C. S.; Zimran, A.; Ewggd *J. Inh. Met. Disease* **2003,** *26* (6), 513-526.
57. Palcic, M. M.; Pierce, M.; Hindsgaul, O. *Neoglycoconjugates, Pt B* **1994,** *247*, 215-227.

58. Ahsen, O. v.; Voigtmann, U.; Klotz, M.; Nifantiev, N.; Schottelius, A.; Ernst, A.; Müller-Tiemann, B.; Parczyk, K. *Anal. Biochem.* **2008,** *372* (1), 96-105.
59. Yan, L. Y.; Smith, D. F.; Cummings, R. D. *Anal. Biochem.* **1994,** *223* (1), 111-118.
60. Lowery, R. G.; Kleman-Leyer, K. *Expert Opin. Ther. Targets* **2006,** *10* (1), 179-190.
61. Gross, H. J.; Sticher, U.; Brossmer, R. *Anal. Biochem.* **1990,** *186* (1), 127-134.
62. Hayashi, Y.; Horibata, Y.; Sakaguchi, K.; Okino, N.; Ito, M. *Anal. Biochem.* **2005,** *345* (2), 181-186.
63. Li, J. J.; Bugg, T. D. H. *Chem. Commun.* **2004,** (2), 182-183.
64. Maeda, T.; Nishimuira, S. I. *Chem.–Eur. J.* **2008,** *14* (2), 478-487.
65. Perrin, J. *J. Phys. Rad.* **1926,** *1*, 390-401.
66. Gross, B. J.; Kraybill, B. C.; Walker, S. *J. Am. Chem. Soc.* **2005,** *127* (42), 14588-14589.
67. Pesnot, T.; Palcic, M. M.; Wagner, G. K. *ChemBioChem 11* (10), 1392-1398.
68. Vocadlo, D. J.; Hang, H. C.; Kim, E. J.; Hanover, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U. S. A.* **2003,** 100 (16), 9116-9121.

Chapter 2

Synthesis of Globotriaosylsphingosine

Introduction

Glycosphingolipids (GSLs) are a ubiquitous class of endogenous compounds that serve many biological functions. The glycosphingolipids that belong to the *globo*-series have the D-Gal-α-D-Gal linkage as a characteristic feature (Figure 1). This galabiose moiety is located in the hydrophilic part of the glycosphingolipid that protrudes from the cell membrane. The galabiose disaccharide is an important recognition site for both toxins and adhesive proteins such as bacterial lectins.[1,2] The class of *globo*-glycosphingolipids include members that serve as tumor-specific antigens,[3] which is exemplified by the overexpression of globotriaosylceramide in Burkitt's lymphoma.[4]

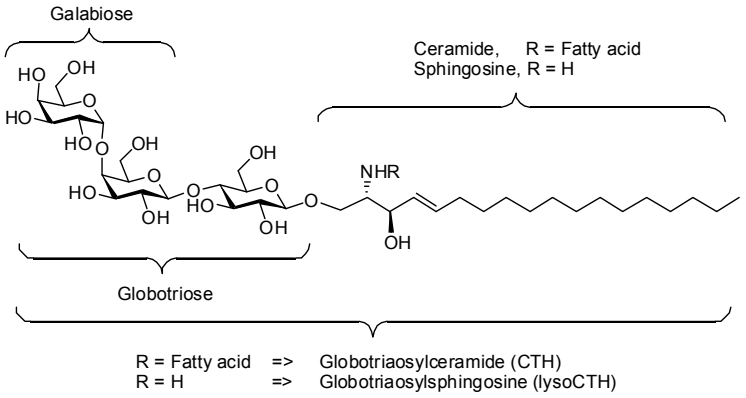


Figure 1. Globotriaosylceramide vs. globotriaosylsphingosine

The metabolism of glycosphingolipids is important and disruption of any of the metabolic transformations due to misfolded or dysfunctional enzymes may ultimately lead to disease processes.[5] For example, an inheritable defect in the gene encoding for α-galactosidase A (GLA) is the underlying cause of Fabry's disease.[6] This genetic defect results in a reduced GLA activity, with a concomitant accumulation of its substrate globotriaosylceramide (CTH).[7] GLA is located in the lysosome of epithelial cells of blood vessels, smooth musculature and the myocardium. Cells with impaired GLA activity increase in size and cause a gradual blockage of the capillaries in affected organs. Although the reduced enzyme activity and accumulation of CTH are undisputed prerequisites for the disease, a correlation between CTH levels and disease manifestations has yet to be observed. Recent findings have shown that the abundance of another glycosphingolipid, namely globotriaosylsphingosine (lysoCTH), which is the deacylated form of CTH has a stronger correlation with the disease manifestations in Fabry patients.[8,9]

This chapter describes a new strategy for the synthesis of lysoCTH, which can be divided in two chemically distinct structures: the hydrophilic globotriose [α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glu] part, and the lipophilic sphingolipid (D-*erythro*-sphingosine) part. Synthesis of the latter entity has been extensively investigated in the literature[10] and the synthesis of a $^{13}C_5$-labeled D-*erythro*-sphingosine will be dealt with in **Chapter 3**.

Results and Discussion

Ever since the first reported synthesis of globotriose by Cox *et al.*[11] and globotriaosylceramide by Shapiro and Acher[12] in 1978, a handful of approaches towards the synthesis of globotriose has been reported in literature. The most commonly used strategy for the assembly of globotriose is to attach a galactose donor to a lactose acceptor (step 1, Figure 2), although condensation of a D-Gal-α-D-Gal disaccharide with a glucose acceptor also has been reported.[13] One-pot procedures[14] using either the "armed-disarmed" concept introduced by Fraser-Reid or *in situ*-removable protecting groups[15] have been utilized. Expeditious routes utilizing fluorous protective groups to minimize the total assembly time have also been published.[16]

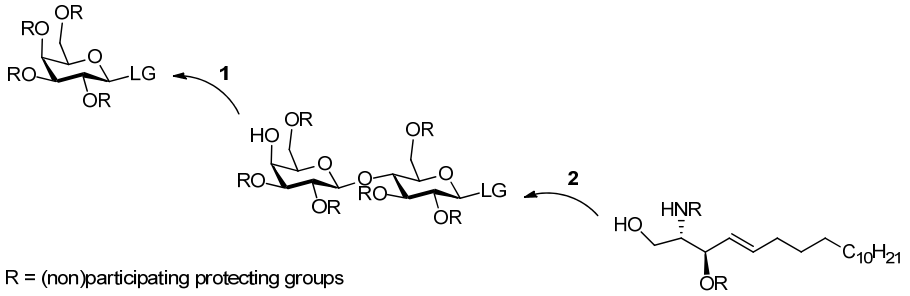
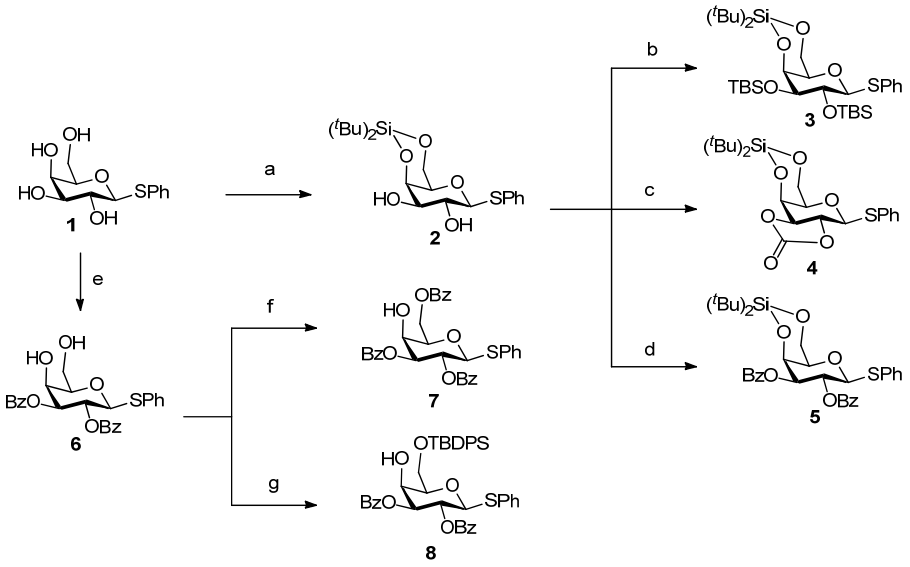


Figure 2. General glycosylation sequence for the synthesis of lysoCTH.

In the development of a robust and high-yielding synthetic route towards lysoCTH, both a high degree of stereoselectivity in glycosylations and minimal protective group manipulations are of importance. First, the stereoselectivity in the glycosylation forming the challenging 1,2-cis linkage of D-Gal-*α*-D-Gal moiety was taken into consideration. Recent literature demonstrates that the presence of a 4,6-*O*-di-*tert*-butylsilylene protecting group in D-galactose donors has a profound effect on the stereoselectivity of its glycosylations.[17] It has been shown that the steric influence of the bulky *tert*-butyl groups (positioned above the anomeric position) can overrule the *trans*-directing properties of a participating group at the C2-OH, giving *α*-selectivity. Furthermore, an advantageous use of protecting groups enabling a mild deprotection sequence is desired. Most syntheses of globotriose make use of a nonparticipating benzyl group at the C2-OH of the galactose donor to achieve high or in some cases complete *α*-selectivity. This strategy requires either a change of protecting groups prior to attachment of the sphingolipid part or deprotection of benzyl protecting groups at the final stage by Birch reduction. It has been reported that Birch reduction of azido-sphingosines requires a Staudinger reduction of the azide, prior to Birch reduction to achieve optimal yields.[18] It was therefore decided to utilize the 4,6-*O*-di-*tert*-butylsilylene protecting group to enable a stereoselective synthesis of CTH without the use of benzyl protecting groups.

The stereochemical outcome of glycosylations forming the D-Gal-α-D-Gal linkage was investigated by the condensation of three different phenyl thiogalactoside donors (**3**–**5**) and acceptors **7** and **8**, which were synthesised according to Scheme 1.

Scheme 1. Synthesis of galactose donors **3**–**5** and acceptors **7** and **8**.

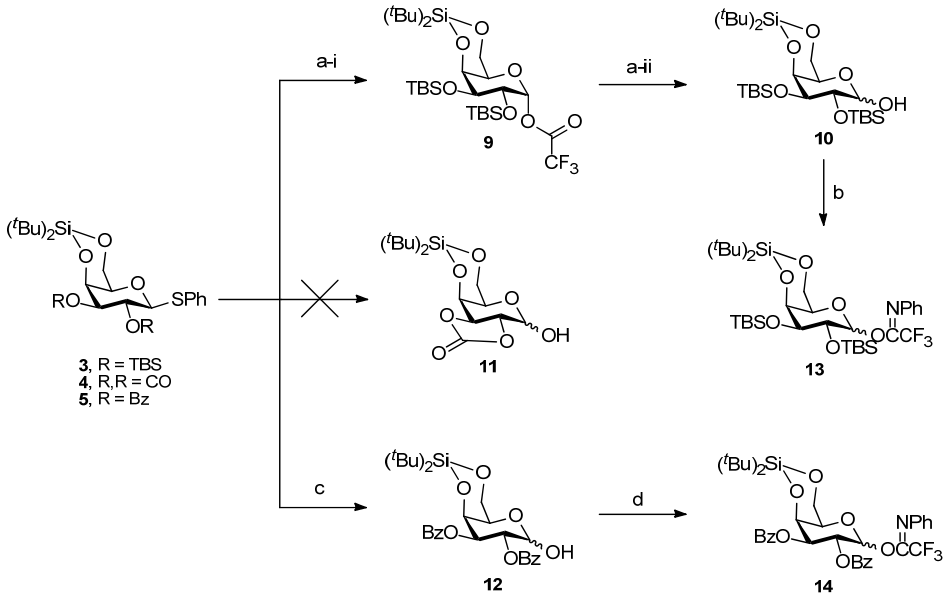


Reagents and conditions: [a] $(^tBu)_2Si(OTf)_2$, pyridine, DMF, −40 °C, 45 min, 93%. [b] TBSOTf, DMAP, pyridine, 0 °C to r.t., 20 h, 83%. [c] $COCl_2$, Et_3N, DCM, 0 °C to r.t., 4 h, 84%. [d] BzCl, pyridine, 3 h, 97%. [e] 3 steps see ref[19] [f] BzCl, pyridine, 0 °C, 1 h, 70%. [g] TBDPSCl, imidazole, DMF, 0 °C to r.t., 20 h, 80%.

Synthesis of all five saccharides could be realized from known phenyl 1-thiogalactoside[20]. Introduction of the 4,6-*O*-di-*tert*-butylsilylene protecting group using di-*tert*-butylsilanediyl bis-triflate[21] in DMF at −40 °C gave **2**, from which donors **3**, **4** and **5** could be synthesized using standard procedures. The silyl ethers in thiodonor **3** were introduced using the very reactive TBS-triflate in pyridine with DMAP as catalyst, since the corresponding TBS-chloride gave incomplete formation of the product. Furthermore, diol **2** was either reacted with phosgene to produce the cyclic carbonate **4** or benzoylated giving thiodonor **5**. Acceptors **7** and **8** were synthesized from phenyl 1-thiogalactoside **1,** which was first transformed into the known galactose diol **6**[19] in three steps as described previously. Regioselective protection of the C6-OH in **6** as TBDPS ether and benzoyl ester produced galactose acceptors **7** and **8**, respectively.

Initially, a chemoselective glycosylation for the condensation of armed thiodonor **3** with disarmed thioacceptor **7** was investigated following a pre-activation strategy according to the procedure of Codée *et al*.[22] This methodology produced a mixture of unidentified products and alternative approaches were therefore investigated.

Scheme 2. Synthesis of galactose hemiacetal donors **10** and **12**, and imidate donors **13** and **14**.



Reagents and conditions: [a] i) NIS, TFA, DCM, 0 °C, 3 h; ii) Et_3N, 0 °C, 20 h, 67%. [b] ClC(NPh)CF_3, Cs_2CO_3, acetone, 0 °C, 2 h, 83%. [c] NIS, TFA, DCM, 0 °C, 4 h, 78%. [d] ClC(NPh)CF_3, Cs_2CO_3, acetone, 0 °C, 3 h, 78%.

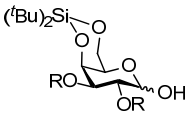
Anomeric hydroxyls can be utilized as donors according to Gin's dehydrative glycosylation methodology.[23] The anomeric thio-functionalities were thus hydrolyzed into the corresponding hemiacetals utilizing the NIS/TFA reagent combination (Scheme 2).[24] Hydrolysis of thiophenyl donor **3** initially formed the anomeric trifluoroacetyl α-D-galactoside **9**, which *in situ* could be converted into the desired

hemiacetal **10**, upon addition of triethylamine. In the formation of **11** was donor **4**, equipped with the 2,3-*trans*-cyclic carbonate hydrolyzed, which resulted in complete degradation of the starting phenyl thio-galactoside, using either NIS/TFA or NBS in wet acetone[25]. This observation is probably a result of the conformational strain present in the tricyclic structure. Satisfactorily, hydrolysis of **5** into **12** occurred smoothly. Hemiacetal donors **10** and **12** were further transformed into the respective *N*-phenyl trifluoroacetimidates[26] **13** and **14**.

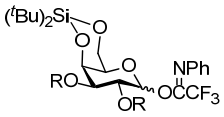
The four galactose donors **10** and **12**–**14** were then evaluated in glycosylation reactions with acceptors **7** and **8** (Table 1). Hemiacetal donors **10** and **12** were both successfully condensed with phenyl 2,3,6-tri-*O*-benzoyl-1-thiogalactoside **7** (entry 1 and 2). Imidate donors **13** and **14** were activated with trifluoromethanesulfonic acid and condensed with acceptors **7** and **8**. Imidate donor **14** effectively produced the desired disaccharide in good yield (entry 4), but concurrent deprotection of the C2,C3-OTBS groups during coupling with **13** resulted in an array of products observed by TLC (entry 3). In all cases complete α-selectivity was observed in the formation of disaccharides **19** and **20**, as proven by the H-1' coupling constant $J_{1'\text{-}2'}$ = ~ 3.5 Hz.

Table 1. Evaluation of coupling conditions for the assembly of globotriose.

Donors

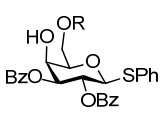


10 R = TBS
12 R = Bz

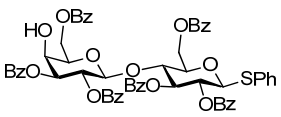


13 R = TBS
14 R = Bz

Acceptors

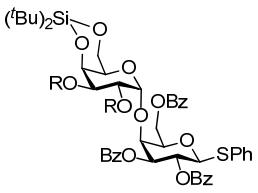


7 R = Bz
8 R = TBDPS

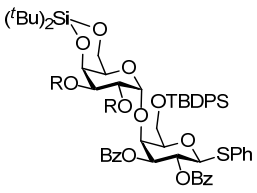


18

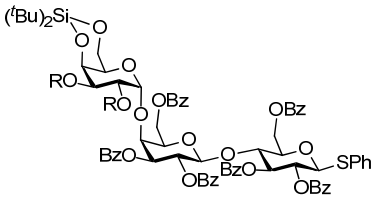
Products



19 R = TBS
20 R = Bz



21 R = TBS
22 R = Bz



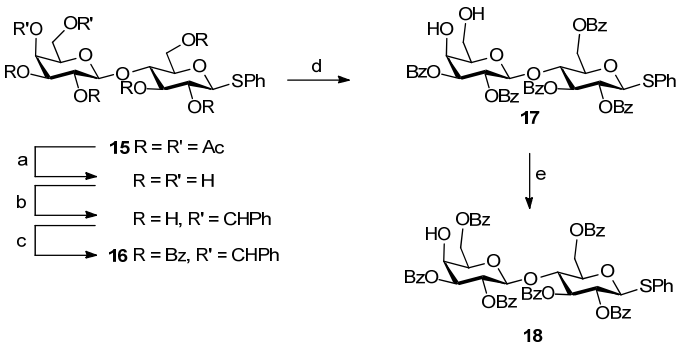
23 R = TBS
24 R = Bz

entry	donor	acceptor	activator	product	yield	entry	donor	acceptor	activator	product	yield
1	**10**	**7**	Ph_2SO, Tf_2O	**19**	90%	6	**12**	**8**	Ph_2SO, Tf_2O	**22**	0%
2	**12**	**7**	Ph_2SO, Tf_2O	**20**	30%	7	**14**	**8**	TfOH	**22**	0%
3	**13**	**7**	TfOH	**19**	0%	8	**10**	**18**	Ph_2SO, Tf_2O	**23**	33%
4	**14**	**7**	TfOH	**20**	75%	9	**12**	**18**	Ph_2SO, Tf_2O	**24**	20%
5	**10**	**8**	Ph_2SO, Tf_2O	**21**	0%	10	**14**	**18**	TfOH	**24**	90%

In contrast to C6-OBz protected acceptor **7**, C6-OTBDPS protected acceptor **8** was unable to react with any of the activated donors (**10**, **12** or **14**). These results clearly show that the steric influence of the C6-OTBDPS in combination with the relatively poor nucleophilicity of the axial C4-OH is detrimental for the acceptor properties of acceptor **8**.

For the assembly of the globotriose was lactose acceptor **18**[27] first synthesized following a modified literature procedure (Scheme 3) by the following sequence of events: 1) deacetylation of phenyl 1-thio-*β*-D-lactoside **15**[28] using Zemplén conditions, 2) protection of the C4',C6'-OH with a benzylidene, 3) perbenzoylation giving fully protected **16**, 4) treatment with TFA giving diol **17** and finally 5) selective benzoylation[29] at the C6'-OH. Condensations towards globotriose with acceptor **18** and donors **10**, **12** or **14** were examined (Table 1, Entry 8–10). Utilizing Gin's dehydrative glycosylation methodology with donors **10** and **12** gave a lower yield with lactose acceptor **18** as compared to galactose acceptor **7**. A similar difference in reactivity between lactose and galactose acceptors has also been observed with a galactosyl bromide donor using an *in situ* anomerization activation protocol.[11] On the other hand, reaction of **18** with imidate donor **14** using a catalytic amount of triflic acid produced the globotriaosyl saccharide **24** in 90% yield. This reaction could be performed on a large (2.5 mmol) scale with identical selectivity and yield, showing the robustness of this particular glycosylation.

Scheme 3. Synthesis of lactose acceptor **18**

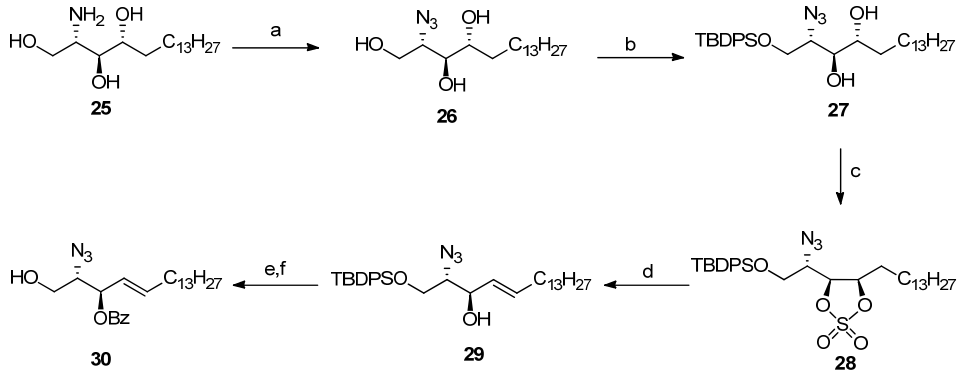


Reagents and conditions: [a] NaOMe, MeOH, r.t., 20 h. [b] $PhCH(OMe)_2$, $pTsOH \cdot H_2O$, MeCN, r.t., 20 h. [c] BzCl, pyridine, r.t., 20 h, 66% (three steps). [d] TFA, DCM, water, 0 °C, 1 h, 92%. [e] HOBt, BzCl, Et_3N, DCM, 0 °C, 5 h, 63%.

There are several reported routes for the synthesis of D-*erythro*-sphingosine and derivates thereof.[30] Following a literature procedure presented by Kim *et al.* synthesis of azido-sphingosine **29** was realized with minor modifications (Scheme 4).[31] Starting from commercially available phytosphingosine **25**, the azide functionality was introduced by a diazotransfer, using imidazole-1-sulfonyl azide as the nitrogen source.[32] After protection of the primary alcohol as a TBDPS-ether giving phytosphingosine **27**, the vicinal alcohols were transformed into a cyclic sulfite by reaction with thionyl chloride. The sulfite was then oxidized using a ruthenium

catalyst and sodium periodate as co-oxidant, forming cyclic sulfate **28**. A regio-selective elimination was performed with DBU to exclusively give the *E*-product and the resulting sulfate ester was hydrolyzed by application of a H_2SO_4/water/THF reagent combination. With azidosphingosine **29** in hand a benzoyl protective group was introduced at C3-OH, followed by deprotection of the primary silyl-ether giving sphingosine acceptor **30**. Unfortunately, concomitant benzoyl-migration to the primary alcohol during desilylation lowered the yield in this step.[33]

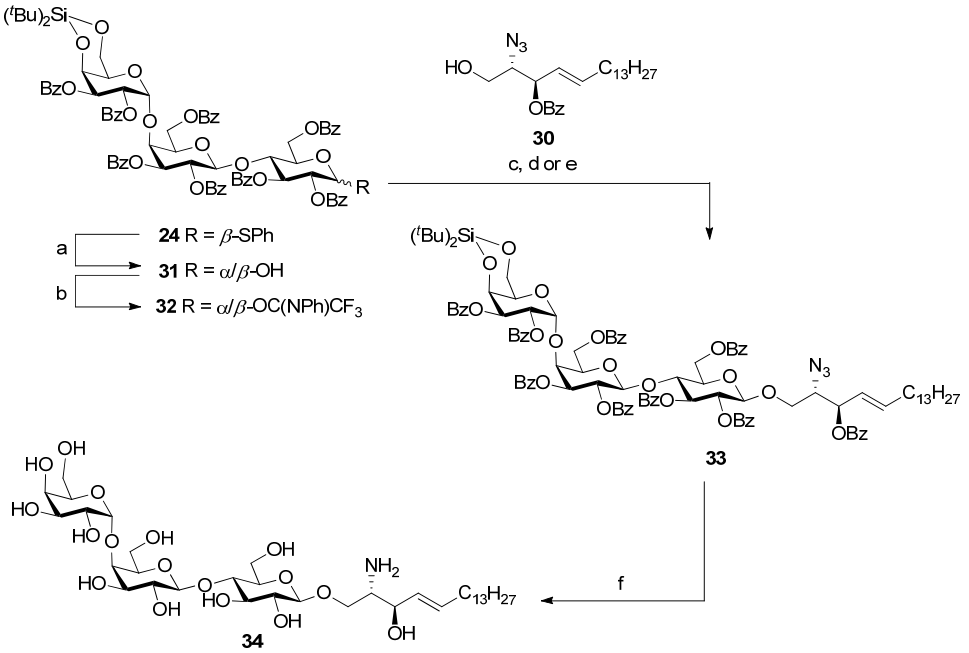
Scheme 4. Synthesis of azido sphingosine **30**



Reagents and conditions: [a] $ImSO_2N_3$, K_2CO_3, $CuSO_4$, MeOH, r.t., 20 h, 98%. [b] TBDPSCl, pyridine, 0 °C to r.t., 20 h, 98%. [c] i) $SOCl_2$, Et_3N, DCM, 0 °C, 1 h; ii) $RuCl_3 \cdot 3H_2O$, $NaIO_4$, CCl_4/MeCN/H_2O, r.t., 2 h, 83%. [d] i) DBU, TBAI, toluene, reflux, 2 h; ii) H_2SO_4/H_2O/THF, r.t., 1 h, 86%. [e] BzCl, pyridine, r.t., 20 h, 93%. [f] TBAF, acetic acid, THF, 0 °C, 1 h, 62%.

The glycosylation of azido-sphingosine **30** was investigated using three different globotriaosyl donors (**24**, **31** and **32**). To this end phenyl 1-thioglobotriaoside **24** was first converted to hemiacetal **31**, which was further transformed into *N*-phenyl trifluoroimidate **32** (Scheme 5). Activation of the thiophenyl donor **24**, using DPS/Tf_2O followed by reaction with azido-sphingosine **30**, produced the desired protected glycosphingolipid **33** in 32% yield. A similar yield (26%) was obtained when hemiacetal donor **31** was activated using Gin's dehydrative methodology in the condensation with **30**. On the other hand, glycosylation of **30** using *N*-phenyl trifluoroacetimidate donor **32** produced the desired product **33** in 80% yield after activation with triflic acid. In contrast to other reports, no orthoester formation was detected using this set of substrates (i.e. C2-OBz as participating group and azido-sphingosine as acceptor).[34] The desired globotriaosylsphingosine was acquired after a three-step deprotection sequence of **33**. Global debenzoylation was performed applying Zemplén conditions, followed by removal of the di-*tert*-butylsilylene protecting group by treatment with hydrogen fluoride in pyridine. The partially deprotected CTH was then purified by column chromatography prior to reduction of the azide with dihydrogen sulfide, to avoid potential acylation of the free amine by methylbenzoate formed during the Zemplén deprotection. RP–HPLC purification using mass-detection produced the target globotriaosylsphingosine **34** in 72% yield.[35]

Scheme 5. Final assembly of globotriaosylsphingosine and deprotection



Reagents and conditions: [a] NIS, TFA, DCM, 0 °C, 3 h, 86%. [b] $ClC(NPh)CF_3$, Cs_2CO_3, acetone, 0 °C, 2 h, 92%. [c] **24**, DPS, Tf_2O, −60 °C, 15 min, then **30**, −60 °C to −30 °C, 32%. [d] **31**, DPS, Tf_2O, −40 °C, 1 h, then **30**, −60 °C to r.t., 6 h, 26%. [e] **32** and **30**, cat.TfOH, 0 °C, 1 h, 80%. [f] i) NaOMe, MeOH, r.t., 3 h; ii) HF·pyridine, r.t., 20 h; iii) pyridine/MeOH/Et_3N, H_2S, r.t., 20 h, 72%.

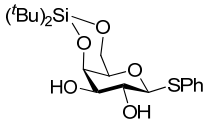
Conclusion

This chapter describes a stereospecific synthesis of globotriaosylsphingosine that is performed in a robust and efficient manner. The optimized assembly strategy utilized an *α*-directing galactosyl imidate, bearing a 4,6-di-*tert*-butylsilylene protective group for the formation of the important D-Gal-*α*-D-Gal glycosidic linkage, which gave access to the phenyl 1-thio-globotrioside with complete stereoselectivity. The globotriaosyl donor was then transformed into the corresponding *N*-phenyl trifluoroimidate donor to achieve optimal yield in the condensation with an azido-sphingosine acceptor. The protected globotriaosyl sphingosine was assembled in 57% yield over four steps from the galactosyl imidate. A mild deprotection sequence produced the desired globotriaosylsphingosine in 72% yield.[36]

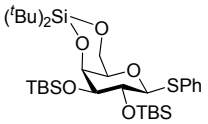
Experimental section

General Procedures and Material: Commercially available reagents and solvents (Acros, Fluka or Merck) were used as received unless stated otherwise. DCM and THF were freshly distilled, before use, over P_2O_5 and Na/benzophenone respectively. Triethylamine was destilled over calcium hydride and stored over KOH. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water was removed from starting compounds by coevaporation with DCE, dioxane and/or toluene. All moisture sensitive reactions were performed under an atmosphere

of argon. Molecular sieves 3Å were flamedried prior to use. Liquid column chromatography was performed using forced flow of the indicated solvent systems on Screening Devices Silica gel 60 (40–63 μm mesh). Size-exclusion chromatography was performed on Sephadex LH20 (eluent MeOH/DCM, 1:1). Analytical TLC was performed on aluminium sheets, pre-coated with silica gel (Merck, silica gel 60, F_{254}). Compounds were visualized with UV absorption (245 nm), by spraying with either 20% H_2SO_4 in ethanol, or ammonium molybdate/cerium sulfate solution [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_6{\cdot}2H_2O$ (10 g/L), 10% sulphuric acid in ethanol], or phosphormolybdic acid in EtOH (150 g/L) followed by charring (~150 °C) or by spraying with an aqueous solution of potassium permanganate [$KMnO_4$ (20 g/L), K_2CO_3 (10 g/L)]. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter (Sodium D-line, λ = 589 nm). ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 (^1H) and 100.6 (^{13}C) MHz or on a Bruker AV 500 MHz spectrometer at 500.0 (^1H) and 125.1 (^{13}C) MHz respectively. Chemical shifts are reported as δ values (ppm) and directly referenced to TMS (0.00 ppm) in $CDCl_3$ or *via* the solvent residual peak (D_2O). Coupling constants (*J*) are given in Hz and all ^{13}C spectra are proton decoupled. NMR assignments were made using COSY and HSQC and in some cases TOCSY experiments. LC-MS analyses were performed on a LCQ Advantage Max (Thermo Finnigan) equipped with a Gemini C_{18} column (Phenomenex, 50 x 4.6 mm, 3μ), utilizing the following buffers: A: H_2O, B: acetonitrile and C: 1.0% $TFA_{(aq)}$. HPLC-MS purifications were performed on a Agilent Technologies 1200 Series automated HPLC system with a Quadropole MS 6130, equipped with a semi-preparative Gemini C_{18} column (Phenomenex, 250 x 10.00, 5 μ). Products were eluted using the following buffers: A: water, B: acetonitrile (HPLC-grade), 5 mL/min. Purified products were lyophilised on a CHRIST ALPHA 2-4 LD_{PLUS}.

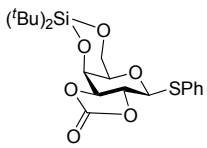


Phenyl 4,6-*O*-(di-tert-butylsilanediyl)-1-thio-*β*-D-galactopyranoside (2) Phenyl 1-thio-*β*-D-galactopyranoside (10.9 g, 40.0 mmol, 1.05 eq) was dried by coevaporation with anhydrous DMF (100 mL) and then dissolved in anhydrous DMF (160 mL). The mixture was cooled to −40 °C before dropwise addition of di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (12.3 mL, 38.1 mmol, 1.0 eq). The reaction was stirred at −40 °C for 30 minutes followed by addition of pyridine (9.24 mL, 114 mmol, 3.0 eq). The reaction was stirred for an additional 15 minutes and then transferred to and extraction funnel with ether (400 mL). The organics were washed with water (400 mL x2) and brine (300 mL). The aqueous layers were extracted with ether (300 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–20% acetone, in DCM) afforded the title compound as a clear viscous oil (14.6 g, 35.4 mmol, 93%). R_f = 0.15 (20% EtOAc in petroleum ether); $[\alpha]_D^{22}$: −63 (C = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.56 – 7.51 (m, 2 H, H_{arom}), 7.33 – 7.24 (m, 3 H, H_{arom}), 4.56 (d, 1 H, *J* = 9.8 Hz, H-1), 4.42 (dd, 1 H, *J* = 3.5, 1.1 Hz, H-4), 4.26 (dd, 1 H, *J* = 12.5, 1.9 Hz, H-6_a), 4.22 (dd, 1 H, *J* = 12.5, 2.2 Hz, H-6_b), 3.76 (dd, 1 H, *J* = 9.8, 9.0 Hz, H-2), 3.55 (dd, 1 H, *J* = 9.0, 3.5 Hz, H-3), 3.45 (m, 1 H, H-5), 3.08 (bs, 2 H, OH), 1.05 (s, 9 H, $CH_{3tBu\text{-}Si}$), 1.04 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 133.2 ($C_{q\text{-}arom}$), 132.3, 128.8, 127.7 (CH_{arom}), 88.8 (C-1), 75.1 (C-5), 75.0 (C-3), 72.5 (C-4), 70.5 (C-2), 67.0 (C-6), 27.5, 27.3 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2, 20.5 ($C_{q\text{-}tBu\text{-}Si}$); IR (neat): 3398, 2934, 2858, 1473, 1162, 1063, 826, 732, 649, 442 cm^{-1}; HRMS Calcd. for $[C_{20}H_{32}O_5SSi + Na]^+$: 435.1632, found 435.1629.

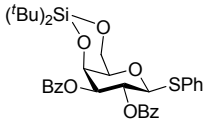


Phenyl 2,3-di-*O*-(*tert*-butyldimethylsilyl)-4,6-*O*-(di-tert-butylsilanediyl)-1-thio-*β*-D-galactopyranoside (3) Phenyl 4,6-*O*-(di-tert-butylsilanediyl)-1-thio-*β*-D-galactopyranoside (1.73 g, 4.2 mmol, 1.0 eq) and DMAP (51 mg, 0.42 mmol, 0.1 eq) were dissolved in pyridine (20 mL) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (2.5 mL, 10.9 mmol, 2.6 eq) was added at 0 °C. The reaction was stirred at 0 °C for 15 minutes and then at room temperature over night. The reaction was concentrated *in vacuo*, diluted with EtOAc

(80 mL) and washed with 1 M HCl (100 mL), sat. aq. $NaHCO_3$ (100 mL) and brine (80 mL). The aqueous layers were extracted with EtOAc (80 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (0–40% DCM in petroleum ether) afforded the title compound as a clear oil (2.22 g, 3.46 mmol, 83%). R_f = 0.33 (40% DCM in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48 (dm, 2 H, *J* = 7.1 Hz, H_{arom}), 7.29 – 7.19 (m, 3 H, H_{arom}), 4.56 (d, 1 H, *J* = 9.4 Hz, H-1), 4.32 (dd, 1 H, *J* = 2.8, 1.0 Hz, H-4), 4.19 (dd, 1 H, *J* = 12.2, 1.9 Hz, H-6_a), 4.15 (dd, 1 H, *J* = 12.2, 2.0 Hz, H-6_b), 4.01 (t, 1 H, *J* = 8.9 Hz, H-2), 3.52 (dd, 1 H, *J* = 8.6, 2.8 Hz, H-3), 3.33 (d, 1 H, *J* = 1.1 Hz, H-5), 1.12 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.04 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.96 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.95 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.26 (s, 3 H, $CH_{3\text{-}TBS}$), 0.15 (s, 3 H, $CH_{3\text{-}TBS}$), 0.12 (s, 3 H, $CH_{3\text{-}TBS}$), 0.10 (s, 3 H, $CH_{3\text{-}TBS}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 135.8, 131.6 (CH_{arom} x2), 128.6 ($C_{q\text{-}arom}$), 126.9 (CH_{arom}), 90.5 (C-1), 77.9 (C-3), 74.7 (C-5), 74.5 (C-4), 70.0 (C-2), 67.3 (C-6), 27.8, 27.4, 26.50, 26.46 ($CH_{3\text{-}tBu\text{-}Si}$ x4), 23.43, 20.7, 18.29, 18.28 ($C_{q\text{-}tBu\text{-}Si}$ x4), −2.0, −3.39, −3.42, −3.7 ($CH_{3\text{-}TBS}$ x4); IR (neat): 2932, 2856, 1473, 1254, 1080, 839, 772 cm^{-1}; HRMS Calcd. for $[C_{32}H_{60}O_5SSi_3 + H]^+$: 641.3542, found 641.3542.

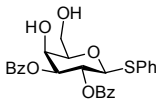


Phenyl 4,6-*O*-(di-tert-butylsilanediyl)-2,3-*O*-carbonyl-1-thio-*β*-D-galactopyranoside (4) Phenyl 4,6-*O*-(di-*tert*-butylsilanediyl)-1-thio-*β*-D-galactopyranoside (1.0 g, 2.5 mmol, 1.0 eq) was dissolved in dry DCM (10 mL) under an atmosphere of argon. Anhydrous triethylamine (1.1 mL, 7.5 mmol, 3.0 eq) was added and the reaction mixture was cooled to 0 °C, before dropwise addition of phosgene (2 M in toluene) (2.5 mL, 5.0 mmol, 2.0 eq). The reaction was stirred for 3 hours, diluted with DCM (20 mL) and quenched by washing with sat. aq. $NaHCO_3$ (30 mL) and brine (25 mL). The aqueous layers were extracted with DCM (30 mL) and the combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by column chromatography (15% EtOAc in petroleum ether) afforded the title compound as a white foam (0.92 g, 2.1 mmol, 84%). R_f = 0.57 (25% EtOAc in petroleum ether); $[\alpha]_D^{22}$: −51 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61 – 7.54 (dm, 2 H, *J* = 6.0 Hz, H_{arom}), 7.38 – 7.30 (m, 3 H, H_{arom}), 4.95 (d, 1 H, *J* = 9.8 Hz, H-1), 4.80 (bs, 1H, H-4), 4.62 (t, 1 H, *J* = 10.5 Hz, H-2), 4.38 – 4.21 (m, 3 H, H-6_a, H-6_b and H-3), 3.58 (bs, 1 H, H-5), 1.06 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.01 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 153.0 (C=O), 133.8 (CH_{arom} x2), 130.9 (C_q), 129.1, 128.7 (CH_{arom} x2), 85.4 (C-1), 83.3 (C-3), 75.7 (C-5), 74.0 (C-2), 69.2 (C-4), 67.0 (C-6), 27.5, 27.3 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2, 20.5 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 2934, 2858, 1815, 1474, 1366, 1221, 1165, 1049, 754 cm^{-1}; HRMS Calcd. for $[C_{21}H_{30}O_6SSi + H]^+$: 439.1605, found 439.1605.

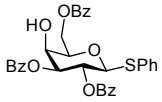


Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilanediyl)-1-thio-*β*-D-galactopyranoside (5) Phenyl 4,6-*O*-(di-tert-butylsilanediyl)-1-thio-*β*-D-galactopyranoside (3.3 g, 8.0 mmol, 1.0 eq) was dissolved in pyridine (20 mL) and benzoyl chloride (2.2 mL, 19 mmol, 2.4 eq) was added at room temperature. The reaction was stirred until TLC showed full conversion to a higher running product and was then quenched with MeOH (1 mL) and concentrated under reduced preassure. The residue was dissolved in EtOAc (50 mL) and washed with 1 M HCl (50 mL), sat. aq. $NaHCO_3$ (50 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by column chromatography (10% EtOAc in petroleum ether) afforded the title compound as a white solid (4.80 g, 7.7 mmol, 97%). R_f = 0.25 (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +121 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 – 7.96 (m, 4 H, H_{arom}), 7.55 – 7.45 (m, 4 H, H_{arom}), 7.42 – 7.35 (m, 4 H, H_{arom}), 7.28 – 7.24 (m, 3 H, H_{arom}), 5.92 (t, 1 H, *J* = 10.0 Hz, H-2), 5.21 (dd, 1 H, *J* = 9.6, 3.2 Hz, H-3), 4.95 (d, 1 H, *J* = 10.0 Hz, H-1), 4.88 (d, 1 H, *J* = 3.2 Hz, H-4), 4.40 – 4.30 (m, 2 H, H-6_a and H-6_b), 3.65 (bs, 1 H, H-5), 1.16 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.96 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.1, 165.4 ($C{=}O_{Bz}$ x2), 133.8 ($C_{q\text{-}arom}$), 133.22, 133.15, 132.4, 129.79 (CH_{arom} x4), 129.77, 129.6 ($C_{q\text{-}arom}$ x2), 129.4, 128.9, 128.37, 128.34, 127.8 (CH_{arom} x5), 87.5 (C-1), 75.4 (C-3), 75.0 (C-5), 70.4 (C-4), 68.1 (C-2), 67.1

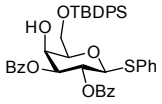
(C-6), 27.48, 27.43 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2, 20.7 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 2934, 2858, 1720, 1273, 1169, 1084, 978, 826, 746 cm^{-1}; HRMS Calcd. for $[C_{34}H_{40}O_7SSi + H]^+$: 621.2337, found 621.2340.



Phenyl 2,3-di-*O*-benzoyl-1-thio-*β*-D-galactopyranoside (6)[19] Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-*β*-D-galactopyranoside (2.1 g, 3.9 mmol, 1.0 eq) was dissolved in AcOH (40 mL) and heated to 80 °C. The reaction was stirred until TLC showed full conversion to a lower running product (~ 3 hours) and was then concentrated *in vacuo*. Purification by column chromatography (20–40% EtOAc in petroleum ether) afforded the title compound as a white solid (1.33 g, 2.8 mmol, 74%). R_f = 0.35 (50% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +105 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (dt, 4 H, *J* = 8.7, 4.4 Hz, H_{arom}), 7.59 – 7.49 (m, 4 H, H_{arom}), 7.40 – 7.24 (m, 7 H, H_{arom}), 5.81 (dd, 1 H, *J* = 10.0, 9.9 Hz, H-2), 5.33 (dd, 1 H, *J* = 9.9, 3.0 Hz, H-3), 4.98 (d, 1 H, *J* = 10.0 Hz, H-1), 4.43 (d, 1 H, *J* = 2.8 Hz, H-4), 4.02 (dd, 1 H, *J* = 11.9, 5.9 Hz, H-6_a), 3.93 (dd, 1 H, *J* = 11.9, 4.5 Hz, H-6_b), 3.82 (t, 1 H, *J* = 5.1 Hz, H-5), 2.65 (bs, 2 H, OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.8, 165.3 ($C{=}O_{Bz}$ x2), 133.4, 133.2, 132.4, 129.83, 129.77 (CH_{arom} x5), 129.3 ($C_{q\text{-}arom}$), 129.0 (CH_{arom}), 128.9 ($C_{q\text{-}arom}$), 128.42, 128.35, 128.1 (CH_{arom} x3), 86.6 (C-1), 78.1 (C-5), 75.5 (C-3), 68.4 (C-4), 67.9 (C-2), 62.7 (C-6); IR (neat): 3470, 3063, 2942, 2878, 1718, 1275, 1093, 1069, 1027, 734, 708, 691 cm^{-1}; HRMS Calcd. for $[C_{26}H_{24}O_7S + H]^+$: 481.1316, found 481.1313.

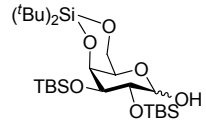


Phenyl 2,3,6-tri-*O*-benzoyl-1-thio-*β*-D-galactopyranoside (7) Phenyl 2,3-di-*O*-benzoyl-1-thio-*β*-D-galactopyranoside (4.8 g, 10 mmol, 1.0 eq) were dissolved in pyridine (30 mL) and cooled to 0 °C followed by addition of benzoyl chloride (1.3 mL, 11 mmol, 1.1 eq). The reaction was stirred for one hour at 0 °C and was then quenched with methanol (1 mL). The mixture was concentrated *in vacuo* and transferred to an extraction funnel using EtOAc (100 mL). The organics were washed with 1 M HCl (100 mL), sat. aq. $NaHCO_3$ (100 mL) and brine (80 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (10–20% EtOAc in petroleum ether) afforded the title compound as a white solid (4.1 g, 7.0 mmol, 70%). R_f = 0.64 (40% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +90 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 (dm, 2 H, *J* = 7.2 Hz, H_{arom}), 7.98 (dm, 2 H, *J* = 7.3 Hz, H_{arom}), 7.95 (dm, 2 H, *J* = 8.1 Hz, H_{arom}), 7.58 (tm, 1 H, *J* = 7.2 Hz, H_{arom}), 7.54 – 7.41 (m, 6 H, H_{arom}), 7.40 – 7.27 (m, 4 H, H_{arom}), 7.23 (m, 1 H, H_{arom}), 7.15 (tm, 2 H, *J* = 7.4 Hz, H_{arom}), 5.84 (t, 1 H, *J* = 9.9 Hz, H-2), 5.41 (dd, 1 H, *J* = 9.9, 3.0 Hz, H-3), 5.00 (d, 1 H, *J* = 10.1 Hz, H-1), 4.70 (dd, 1 H, *J* = 11.7, 5.3 Hz, H-6_a), 4.64 (dd, 1 H, *J* = 11.7, 6.6 Hz, H-6_b), 4.43 (m, 1 H, H-4), 4.15 (bt, 1 H, *J* = 6.1 Hz, H-5), 2.78 (bd, 1 H, *J* = 5.2 Hz, OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.4, 165.8, 165.3 ($C{=}O_{Bz}$ x3), 133.5, 133.3, 133.2 (CH_{arom} x3), 132.9 ($C_{q\text{-}arom}$), 132.2, 129.81, 129.76, 129.75 (CH_{arom} x4), 129.5, 129.3, 128.84 ($C_{q\text{-}arom}$ x3), 128.82, 128.41, 128.40, 128.3, 127.9 (CH_{arom} x5), 86.8 (C-1), 76.3 (C-5), 75.2 (C-3), 67.9 (C-4), 67.6 (C-2), 63.5 (C-6); IR (neat): 3483, 3078, 2930, 2856, 1720, 1275, 1105, 1093, 1069, 1026, 740 cm^{-1}; HRMS Calcd. for $[C_{42}H_{42}O_7SSi + Na]^+$: 741.2313, found 741.2312.

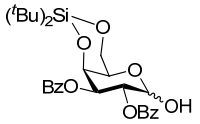


Phenyl 2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-*β*-D-galactopyranoside (8) Phenyl 2,3-di-*O*-benzoyl-1-thio-*β*-D-galactopyranoside (1.8 g, 3.7 mmol, 1.0 eq) and imidazole (0.38 g, 5.6 mmol, 1.5 eq) were dissolved in anhydrous DMF (20 mL) and cooled to 0 °C. *Tert*-butyldiphenylchlorosilane (1.1 g, 4.1 mmol, 1.1 eq) was dissolved in anhydrous DMF (10 mL) and added to the cooled solution. The reaction was stirred at ambient temperature over night and was quenched with methanol (1 mL). The mixture was transferred to an extraction funnel using EtOAc (100 mL), washed with water (100 mL x 2) and brine (80 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–10% EtOAc in

petroleum ether) afforded the title compound as a white solid (2.2 g, 3.0 mmol, 80%). R_f = 0.60 (20% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +70 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (dm, 4 H, *J* = 7.4 Hz, H_{arom}), 7.77 – 7.68 (m, 4 H, H_{arom}), 7.52 – 7.29 (m, 14 H, H_{arom}), 7.26 – 7.19 (m, 3 H, H_{arom}), 5.83 (t, 1 H, *J* = 9.9 Hz, H-2), 5.33 (dd, 1 H, *J* = 9.9, 2.9 Hz, H-3), 4.95 (d, 1 H, *J* = 9.9 Hz, H-1), 4.49 (m, 1 H, H-4), 4.05 (dd, 1 H, *J* = 10.9, 5.0 Hz, H-6_a), 4.00 (dd, 1 H, *J* = 10.9, 4.7 Hz, H-6_b), 3.80 (t, 1 H, *J* = 4.7 Hz, H-5), 3.02 (bd, 1 H, *J* = 3.7 Hz, OH), 1.08 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.9, 165.2 ($C{=}O_{Bz}$ x2), 135.6, 135.5, 134.7, 133.3, 133.1 (CH_{arom} x5), 132.71, 132.65, 132.42 ($C_{q\text{-}arom}$ x3), 132.38, 129. 9, 129.8, 129.7 (CH_{arom} x4), 129.5, 129.1 ($C_{q\text{-}arom}$ x2), 128.8, 128.33, 128.28, 127.82, 127.80, 127.6 (CH_{arom} x6), 86.7 (C-1), 77.9 (C-5), 75.7 (C-3), 68.5 (C-4), 67.9 (C-2), 64.0 (C-6), 26.8 ($CH_{3\text{-}tBu\text{-}Si}$), 19.1 ($C_{q\text{-}tBu\text{-}Si}$); IR (neat): 3483, 3078, 2930, 2856, 1720, 1275, 1105, 1093, 1069, 1026, 740 cm^{-1}; HRMS Calcd. for $[C_{42}H_{42}O_7SSi + Na]^+$: 741.2313, found 741.2312.

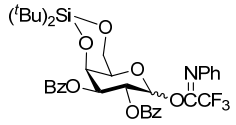


2,3-Di-*O*-(*tert*-butyldimethylsilyl)-4,6-*O*-(di-*tert*-butylsilanediyl)-*α/β*-D-galactopyranose (10) Phenyl 2,3-di-*O*-(*tert*-butyldimethylsilyl)-4,6-*O*-(di-*tert*-butylsilanediyl)-1-thio-*β*-D-galactopyranoside (1.9 g, 3.0 mmol, 1.0 eq) was dissolved in DCM (30 mL). *N*-iodosuccinimide (1.3 g, 5.9 mmol, 2.0 eq) was added at 0 °C, before addition of trifluoroacetic acid (0.23 mL, 3.0 mmol, 1.0 eq). The reaction was left stirring at 0 °C under exposure to the atmosphere. Initially TLC showed full conversion to a higher running product (anomeric TFA-adduct).§ Triethylamine (1.3 mL, 8.9 mmol, 3.0 eq) was added and the reaction showed full conversion to a lower running product after 5 hours. The mixture was then transferred to an extraction funnel with EtOAc (70 mL). The organics were washed with sodium thiosulfate (20% aq.) (100 mL), sat. aq. $NaHCO_3$ (100 mL) and brine (80 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–20% EtOAc in petroleum ether) afforded the title compound as a clear oil (1.1 g, 2.0 mmol, 67%). R_f = 0.30 (10% EtOAc in petroleum ether); NMR assignment of major isomer (*α*) ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.18 (d, 1 H, *J* = 3.5 Hz, H-1), 4.30 (d, 1 H, *J* = 2.9 Hz, H-4), 4.23 (dd, 1 H, *J* = 12.4, 1.7 Hz, H-6_a), 4.17 (dd, 1 H, *J* = 12.4, 1.7 Hz, H-6_b), 4.07 (dd, 1 H, *J* = 9.3, 3.5 Hz, H-2), 3.89 (bs, 1 H, H-5), 3.79 (dd, 1 H, *J* = 9.3, 2.9 Hz, H-3), 2.99 (bs, 1 H, OH), 1.04 (bs, 18 H, $CH_{3tBu\text{-}Si}$ x 2), 0.94 (s, 9 H, $CH_{3tBu\text{-}Si}$), 0.92 (s, 9 H, $CH_{3tBu\text{-}Si}$), 0.13 (s, 3 H, $CH_{3\text{-}TBS}$), 0.11 (s, 3 H, $CH_{3\text{-}TBS}$), 0.10 (s, 6 H, $CH_{3\text{-}TBS}$ x 2); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 94.0 (C-1), 74.6 (C-3), 71.3 (C-4), 70.1 (C-5), 67.9 (C-2), 67.4 (C-6), 27.42, 27.35, 26.04, 25.99 ($CH_{3\text{-}tBu\text{-}Si}$ x4), 23.4, 20.7, 18.07, 18.05 ($C_{q\text{-}tBu\text{-}Si}$ x4), −4.0, −4.1, −4.3, −4.8 ($CH_{3\text{-}TBS}$ x4); IR (neat): 2932, 2858, 1472, 1253, 1102, 833, 774, 648 cm^{-1}; HRMS Calcd. for $[C_{26}H_{56}O_6Si_3 + H]^+$: 549.3458, found 549.3453. § The anomeric TFA-adduct could be isolated: **Trifluoroacetyl 2,3-di-*O*-(*tert*-butyldimethylsilyl)-4,6-*O*-(di-*tert*-butylsilandiyl)-1-thio-*α*-D-galacto-pyranoside (9)** R_f = 0.95 (10% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.38 (d, 1 H, *J* = 3.3 Hz, H-1), 4.45 (d, 1 H, *J* = 2.2 Hz, H-4), 4.34 (dd, 1 H, *J* = 9.5, 3.3 Hz, H-2), 4.27 (d, 1 H, *J* = 12.7 Hz, H-6_a), 4.20 (d, 1 H, *J* = 12.7 Hz, H-6_b), 3.91 (dd, 1 H, *J* = 9.5, 2.7 Hz, H-3), 3.85 (bs, 1 H, H-5), 1.09 (bs, 18 H $CH_{3tBu\text{-}Si}$ x2), 0.98 (s, 9 H, $CH_{3tBu\text{-}Si}$), 0.90 (s, 9 H, $CH_{3tBu\text{-}Si}$), 0.15 (s, 3 H, $CH_{3\text{-}TBS}$), 0.14 (s, 6 H, $CH_{3\text{-}TBS}$ x2), 0.13 (s, 3 H, $CH_{3\text{-}TBS}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 156.2 (q, *J* = 42.5 Hz, C=O), 114.6 (q, *J* = 286.1 Hz, CF_3), 98.0 (C-1), 74.2 (C-3), 71.11 (C-5), 71.06 (C-4), 68.0 (C-2), 66.6 (C-6), 27.4, 27.3, 26.1, 25.8 ($CH_{3tBu\text{-}Si}$ x4), 23.4, 20.8, 18.1, 17.8 ($C_{q\text{-}tBu\text{-}Si}$ x4), -4.2, -4.4, -4.5, -4.7 ($CH_{3\text{-}TBS}$ x4).

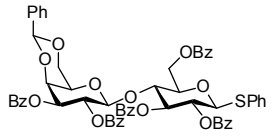


2,3-Di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-*α/β*-D-galactopyranose (12) Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-1-thio-*β*-D-galactopyranoside (5.5 g, 8.9 mmol, 1.0 eq) was dissolved in DCM (85 mL). *N*-iodosuccinimide (4.0 g, 18 mmol, 2.0 eq) was added at 0 °C followed by addition of trifluoroacetic acid (0.68 mL, 8.9 mmol, 1.0 eq). The reaction was left stirring under exposure to the atmosphere

until TLC showed full conversion. The mixture was then transferred to an extraction funnel with EtOAc (150 mL) and washed with sodium thiosulfate (20% aq.) (200 mL), sat. aq. $NaHCO_3$ (200 mL) and brine (150 mL). The aqueous layers were extracted with EtOAc (150 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (0–10% diethylether, 40% DCM in petroleum ether) afforded the title compound as a white solid (3.7 g, 6.9 mmol, 78%). R_f = 0.25 (20% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 – 7.97 (m, 4 H, CH_{arom}), 7.56 – 7.49 (m, 2 H, CH_{arom}), 7.42 – 7.35 (m, 4 H, CH_{arom}), 5.77 (dd, 0.7 H, *J* = 10.5, 3.5 Hz, H-2$_\alpha$), 5.72 (m, 0.7 H, H-1$_\alpha$), 5.66 (m, 1 H, H-3$_\alpha$ and H-2$_\beta$), 5.31 (dd, 0.3 H, *J* = 10.2, 3.2 Hz, H-3$_\beta$), 4.91 – 4.85 (m, 1 H, H-4$_\alpha$ and H-1$_\beta$), 4.83 (d, 0.3 H, *J* = 3.0 Hz, H-4$_\beta$), 4.38 – 4.31 (m, 1.3 H, H-6$_{a\text{-}\alpha}$, H-6$_{a\text{-}\beta}$ and H-6$_{b\text{-}\alpha}$), 4.22 (dd, 0.7 H, *J* = 12.6, 1.6 Hz, H-6$_{b\text{-}\beta}$), 4.19 (m, 0.7 H, H-5$_\alpha$), 3.68 (m, 0.3 H, H-5$_\beta$), 3.63 (d, 0.3 H, OH$_\beta$), 2.81 (s, 0.7 H, OH$_\alpha$), 1.15 (s, 2.7 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 1.13 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$), 0.98 (s, 6.3 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.4, 166.21, 166.15, 166.10 (C=O, Bz x 4), 133.5 , 133.33, 133.29, 133.1, 129.9, 129.8, 129.74, 129.71 (CH_{arom} x8), 129.42, 129.40, 129.1 ($C_{q\text{-}arom}$ x3), 128.43, 128.37 (CH_{arom} x2), 96.3 (C-1$_\beta$), 91.2 (C-1$_\alpha$), 73.4 (C-3$_\beta$), 71.9 (C-2$_\beta$), 71.7 (C-5$_\beta$), 71.3 (C-4$_\alpha$), 70.6 (C-3$_\alpha$), 70.5 (C-4$_\beta$), 68.9 (C-2$_\alpha$), 67.04 (C-6$_\alpha$), 67.03 (C-5$_\alpha$), 66.9 (C-6$_\beta$), 27.5, 27.43, 27.36, 27.2 (CH_3, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$ x4), 23.28, 23.26, 20.8, 20.7 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$ x4); IR (neat): 3473, 2935, 2860, 1719, 1267, 1100, 1073, 997, 708, 441 cm^{-1}; HRMS Calcd. for $[C_{28}H_{36}O_8Si + H]^+$: 529.2252, found 529.2249.

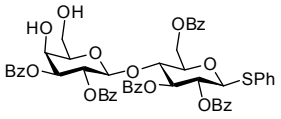


2,3-Di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)- *α/β*-D-galactopyranoside (14) 2,3-di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-α/*β*-D-galactopyranose (1.9 g, 3.5 mmol, 1.0 eq) was dissolved in acetone (20 mL) and cooled to 0 °C. Cesium carbonate (1.7 g, 5.3 mmol, 1.5 eq) was added followed by chloro *N*-phenyl-trifluoroimidate (1.1 g, 5.3 mmol, 1.5 eq) and the reaction was stirred at 0 °C for two hours. The reaction mixture was filtrated and concentrated *in vacuo*. Purification by column chromatography, which initially was neutralized by running an eluent of 3% Et_3N in petroleum ether (100 mL) through the column, (0–5% EtOAc, 20% DCM in petroleum ether) produced the title compound as a white solid (1.9 g, 2.7 mmol, 78%). R_f = 0.64 and 0.58 (10% EtOAc and 20% DCM in petroleum ether); NMR assignment of major isomer (*α*) ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 – 7.98 (m, 4 H, H_{arom}), 7.61 – 7.50 (m, 2 H, H_{arom}), 7.46 – 7.35 (m, 4 H, H_{arom}), 7.12 (t, 2 H, *J* = 7.7 Hz, $H_{arom\text{-}NPh}$), 7.00 (t, 1 H, *J* = 7.7 Hz, $H_{arom\text{-}NPh}$), 6.87 (bs, 1H, H-1), 6.40 (bs, 2 H, $H_{arom\text{-}NPh}$), 6.02 (bd, 1 H, *J* = 9.2 Hz, H-2), 5.65 (dd, 1 H, *J* = 10.6, 3.3 Hz, H-3), 5.00 (s, 1 H, H-4), 4.40 – 4.26 (m, 2 H, H-6$_a$ and H-6$_b$), 4.15 (bs, 1 H, H-5), 1.13 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.98 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.1, 165.6 ($C{=}O_{Bz}$ x2), 143.0 ($C{=}N_{NPh}$), 133.5, 133.3, 129.8, 129.7 (CH_{arom} x4), 129.5, 129.0 ($C_{q\text{-}arom}$ x2), 128.6, 128.5, 128.4 (CH_{arom} x3), 124.2 (C-1), 119.1 ($CH_{arom\text{-}NPh}$), 70.74 (C-3), 70.65 (C-4), 69.8 (C-5), 67.2 (C-2), 66.6 (C-6), 27.5, 27.2 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.3, 20.8 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 2948, 2862, 1732, 1276, 1208, 1119, 994, 786, 707, 443 cm^{-1}; HRMS Calcd. for $[C_{36}H_{40}F_3NO_8Si + H]^+$: 700.2548, found 700.2549.



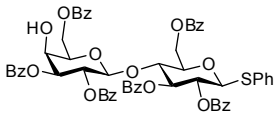
Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-benzylidene-*β*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside (16) Phenyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galacto-pyranosyl)-1-thio-*β*-D-glucopyranoside (17 g, 23.0 mmol, 1.0 eq) was dissolved in methanol (250 mL) and sodium methoxide (30% in methanol) (0.64 mL, 4.6 mmol, 0.20 eq) was added at ambient temperature. The reaction was run over night giving full conversion to a lower running product (TLC; 30% MeOH in EtOAc, R_f = 0.32). The reaction was neutralized with Amberlite H^+, filtrated and concentrated *in vacuo*, producing a white solid that was used without further purification. The solids were dried by means of a vacuum pump over night. The crude phenyl 4-*O*-(*β*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside was then suspended in

anhydrous acetonitrile (300 mL) and placed under argon. Benzaldehyde dimethylacetal (5.2 mL, 35 mmol, 1.5 eq) was added followed by *p*-TsOH·H_2O (0.22 g, 1.2 mmol, 0.05 eq). The reaction was left stirring at ambient temperature over night, giving a clear solution (TLC; 10% MeOH in EtOAc, R_f = 0.33). The reaction was quenched by addition of triethylamine (1.6 mL, 12 mmol, 0.5 eq) and was then concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL) and washed with sat. aq. $NaHCO_3$ (200 mL) and brine (200 mL). The aqueous layers were extracted with EtOAc (200 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude phenyl 4-*O*-(4,6-benzylidene-*β*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside was dissolved in anhydrous pyridine (150 mL) followed by addition of benzoyl chloride (16 mL, 140 mmol, 6.0 eq) at ambient temperature. The reaction was stirred over night, quenched by addition of MeOH (2 mL) and concentrated *in vacuo*. Crystallization from DCM:petroleum ether produced the title compound as a white cotton like solid (10.6 g, 10 mmol, 44%). The mother liquor was then purified by column chromatography (0–10% EtOAc, 40% DCM in petroleum ether) for additional product (5.2 g, 5.0 mmol, 22%) in a total yield of 66% over three steps. R_f = 0.80 (50 % EtOAc in petroleum ether). $[\alpha]_D^{22}$: +107 (C = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 – 7.86 (m, 10 H, H_{arom}), 7.60 (tm, 1 H, *J* = 7.4 Hz, H_{arom}), 7.51 (tm, 1 H, *J* = 7.4 Hz, H_{arom}), 7.47 – 7.41 (m, 4 H, H_{arom}), 7.40 – 7.35 (m, 4 H, H_{arom}), 7.34 – 7.26 (m, 10 H, H_{arom}), 7.21 – 7.13 (m, 3 H, H_{arom}), 7.04 (tm, 2 H, *J* = 7.6 Hz, H_{arom}), 5.84 (t, 1 H, *J* = 9.1 Hz, H-3), 5.78 (dd, 1 H, *J* = 10.4, 7.9 Hz, H-2′), 5.31 (t, 1 H, *J* = 9.7 Hz, H-2), 5.28 (s, 1 H, $CH_{benzylidene}$), 5.16 (dd, 1 H, *J* = 10.4, 3.6 Hz, H-3′), 4.91 (d, 1 H, *J* = 10.0 Hz, H-1), 4.83 (d, 1 H, *J* = 7.9 Hz, H-1′), 4.66 (dd, 1 H, *J* = 11.9, 1.9 Hz, H-6_a), 4.39 (dd, 1 H, *J* = 11.9, 5.1 Hz, H-6_b), 4.31 (d, 1 H, *J* = 3.3 Hz, H-4′), 4.13 (dd, 1 H, *J* = 9.7, 9.1 Hz, H-4), 3.89 (ddd, 1 H, *J* = 9.7, 5.1, 1.9, Hz, H-5), 3.71 (d, 1 H, *J* = 11.5 Hz, H-6′$_a$), 3.56 (d, 1 H, *J* = 11.5 Hz, H-6′$_b$), 2.99 (m, 1 H, H-5′); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.1, 165.5, 165.3, 165.0, 164.8 ($C{=}O_{Bz}$ x5), 137.4 ($C_{q\text{-}arom}$), 133.3, 133.2, 133.1, 133.03, 133.01 (CH_{arom} x5), 131.6 ($C_{q\text{-}arom}$), 129.88, 129.86, 129.7, 129.6 (CH_{arom} x4), 129.5, 129.3, 128.92, 128.87 ($C_{q\text{-}arom}$ x4), 128.8, 128.7, 128.32, 128.26, 128.1, 127.9, 126.3 (CH_{arom} x7), 101.5 (C-1′), 100.6 ($CH_{benzylidene}$), 85.6 (C-1), 76.8 (C-4 and C-5), 75.0 (C-3), 73.0 (C-4′), 72.6 (C-3′), 70.8 (C-2), 69.5 (C-2′), 67.9 (C-6′), 66.5 (C-5′), 62.6 (C-6); IR (neat): 3070, 2942, 2862, 1717, 1452, 1276, 1113, 1027, 706 cm^{-1}; HRMS Calcd. for $[C_{60}H_{50}O_{15}S + Na]^+$: 1065.2763, found 1065.2765.

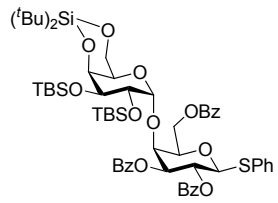


Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-*β*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside (17) Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-benzylidene-*β*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside (6.8 g, 6.5 mmol, 1.0 eq) was dissolved in DCM (250 mL) and cooled to 0 °C. Trifluoroacetic acid (2.5 mL, 33 mmol, 5.0 eq) was added followed by water (1 mL). The reaction was run until TLC showed full conversion to a lower running spot. The reaction mixture was transferred to an extraction funnel and washed wit sat. aq. $NaHCO_3$ (200 mL) and brine (150 mL). The aqueous layers were extracted with EtOAc (200 mL) and the combined organics were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by column chromatography (5–20% EtOAc, 40% DCM in petroleum ether) produced the title compound as a white solid (5.7 g, 6.0 mmol, 92%). R_f = 0.35 (50% EtOAc in petroleum ether). $[\alpha]_D^{22}$: +78 (C = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.01 – 7.97 (m, 2 H, H_{arom}), 7.96 – 7.92 (m, 4 H, H_{arom}), 7.92 – 7.88 (m, 4 H, H_{arom}), 7.61 (tm, 1 H, *J* = 7.4 Hz, H_{arom}), 7.56 – 7.50 (m, 2 H, H_{arom}), 7.49 – 7.43 (m, 3 H, H_{arom}), 7.42 – 7.36 (m, 6 H, H_{arom}), 7.34 – 7.28 (m, 3 H, H_{arom}), 7.23 – 7.15 (m, 3 H, H_{arom}), 7.06 (tm, 2 H, *J* = 7.6 Hz, H_{arom}), 5.76 (t, 1 H, *J* = 8.9 Hz, H-3), 5.73 (dd, 1 H, *J* = 10.4, 7.9 Hz, H-2′), 5.39 (t, 1 H, *J* = 9.6 Hz, H-2), 5.09 (dd, 1 H, *J* = 10.4, 3.1 Hz, H-3′), 4.92 (d, 1 H, *J* = 9.9 Hz, H-1), 4.78 (d, 1 H, *J* = 7.9 Hz, H-1′), 4.65 (dd, 1 H, *J* = 11.9, 1.9 Hz, H-6_a), 4.43 (dd, 1 H, *J* = 11.9, 5.7 Hz, H-6_b), 4.20 (d, 1 H, *J* = 3.1 Hz, H-4′), 4.10 (dd, 1 H, *J* = 9.4, 8.9 Hz, H-4), 3.89 (ddd, 1 H, *J* = 9.4, 5.7, 1.9, Hz, H-5), 3.41 – 3.33 (m, 2 H, H-5′ and H-6′$_a$), 3.26 (dd, 1 H, *J* = 11.8, 5.0 Hz, H-6′$_b$), 1.70 (bs, 2 H, OH x2); ^{13}C NMR (101 MHz,

$CDCl_3$) δ: 165.82, 165.75, 165.4, 165.2, 165.0 ($C{=}O_{Bz}$ x5), 133.5, 133.32, 133.25, 133.2, 133.0 (CH_{arom} x5), 131. 8 ($C_{q\text{-}arom}$), 129.85, 129.81, 129.74 (CH_{arom} x3), 129.70 ($C_{q\text{-}arom}$), 129.64, 129.55 (CH_{arom} x2), 129.2, 129.0, 128.80 ($C_{q\text{-}arom}$ x3), 128.77, 128.6, 128.42, 128.39, 128.38, 128.36, 128.1 (CH_{arom} x7), 101.3 (C-1′), 85.8 (C-1), 76.9 (C-5), 76.5 (C-4), 74.6 (C-3), 74.3 (C-5′), 74.2 (C-3′), 70. 5 (C-2), 69.7 (C-2′), 68.1 (C-4′), 62.9 (C-6), 62.4 (C-6′); IR (neat): 3490, 3064, 2948, 2872, 1722, 1269, 1069, 1027, 707 cm^{-1}; HRMS Calcd. for $[C_{53}H_{46}O_{15}S + Na]^+$: 977.2450, found 977.2448.

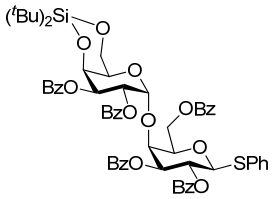


Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-*β*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside (18) Benzoyl chloride (0.511 mL, 4.40 mmol, 1.1 eq) was added dropwise to a solution of hydroxybenzotriazole (0.60 g, 4.4 mmol, 1.1 eq) and triethylamine (0.62 mL, 4.4 mmol, 1.1 eq) in anhydrous DCM (16 mL). When TLC showed full conversion to the activated ester, phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-*β*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside (3.8 g, 4.0 mmol, 1.0 eq), dissolved in DCM (5 mL) was added followed by triethylamine (0.62 mL, 4.4 mmol, 1.1 eq). The reaction was stirred until no more conversion to product was seen and the mixture was transferred to an extraction funnel using EtOAc (80 mL). The organics were washed with sat. aq. $NaHCO_3$ (100 mL) and brine (80 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–15% EtOAc, 40% DCM in petroleum ether) produced the title compound as a white solid (2.68 g, 2.53 mmol, 63%). R_f = 0.12 (20% EtOAc in petroleum ether). $[\alpha]_D^{22}$: +55 (C = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.02 – 7.87 (m, 12 H, H_{arom}), 7.62 – 7.53 (m, 2 H, H_{arom}), 7.52 – 7.35 (m, 11 H, H_{arom}), 7.32 – 7.27 (m, 3 H, H_{arom}), 7.24 – 7.17 (m, 5 H, H_{arom}), 7.12 – 7.07 (m, 2 H, H_{arom}), 5.85 – 5.75 (m, 2 H, H-3 and H-2′), 5.43 (t, 1 H, *J* = 9.7 Hz, H-2), 5.18 (dd, 1 H, *J* = 10.4, 3.5 Hz, H-3′), 4.98 (d, 1 H, *J* = 10.0 Hz, H-1), 4.79 (d, 1 H, *J* = 7.9 Hz, H-1′), 4.62 (d, 1 H, *J* = 11.8 Hz, H-6_a), 4.50 (dd, 1 H, *J* = 11.8, 5.3 Hz, H-6_b), 4.20 (m, 1 H, H-4′), 4.17 – 4.07 (m, 2 H, H-4 and H-$6'_a$), 3.97 (m, 1 H, H-5′), 3.76 (t, 1 H, *J* = 7.6 Hz, H-5), 3.62 (dd, 1 H, *J* = 11.2, 6.6 Hz, H-$6'_b$), 2.91 (bs, 1 H, OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.9, 165.75, 165.73, 165.6, 165.2, 165.0 ($C{=}O_{Bz}$ x6), 133.34, 133.25, 133.18, 133.15, 133.1, 132.6 (CH_{arom} x6), 131.9 ($C_{q\text{-}arom}$), 129.82, 129.80, 129.59, 129.56 (CH_{arom} x4), 129.48, 129.46, 129.1, 128.8 ($C_{q\text{-}arom}$ x4), 128.74 (CH_{arom}), 128.67 ($C_{q\text{-}arom}$), 128.5, 128.4, 128.2, 127.9 (CH_{arom} x4), 101.2 (C-1′), 85.6 (C-1), 76.9 (C-5′), 76.3 (C-4), 74.2 (C-3′), 74.09 (C-3), 72.7 (C-5), 70.4 (C-2), 69.6 (C-2′), 66.7 (C-4′), 62.8 (C-6′), 62.0 (C-6); IR (neat): 3487, 3062, 2950, 2868, 1722, 1265, 1093, 1069, 1026, 706 cm^{-1}; HRMS Calcd. for $[C_{60}H_{50}O_{16}S + Na]^+$: 1081.2712, found 1081.2709.



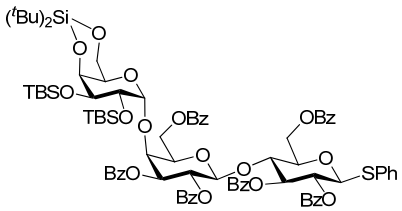
Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-bis-*O*-(*tert*-butyldimethylsilyl)-4,6-*O*-(di-tert-butylsilanediyl)-*α*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside (19) Galactose hydroxyl donor **(10)** (66 mg, 0.12 mmol, 1.2 eq), diphenylsulfoxide (54 mg, 0.26 mmol, 2.6 eq) and 2,4,6-tris-*tert*-butylpyrimidine (75 mg, 0.30 mmol, 3.0 eq), were dissolved in anhydrous DCM (4 mL), under an atmosphere of argon. Activated molsieves (3Å) were added and the mixture was stirred for one hour at ambient temperature. The solution was then cooled to −60 °C and trifluoromethanesulfonic anhydride (22.0 µL, 0.13 mmol, 1.3 eq) was added. The mixture was warmed to −40 °C and stirred at this temperature for an hour. The reaction was then cooled to −60 °C and galactose acceptor **(7)** (59 mg, 0.10 mmol, 1.0 eq) was added as a solution in anhydrous DCM (2 mL). The reaction was stirred for three hours while the temperature was allowed to reach ambient temperature and was then quenched by addition of anhydrous triethylamine (0.14 mL, 1.0 mmol, 10 eq). The mixture was transferred to an extraction funnel

using EtOAc (40 mL) and the organics were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtrated and concentrated *in vacuo*. Purification by column chromatography (10−30% diethylether in petroleum ether) produced the title compound as a white solid (100 mg, 90 µmol, 90%). R_f = 0.31 (15% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +54 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 – 8.09 (m, 2 H, H_{arom}), 8.07 – 8.02 (m, 2 H, H_{arom}), 7.95 – 7.91 (m, 2 H, H_{arom}), 7.61 (m, 1 H, H_{arom}), 7.54 – 7.45 (m, 7 H, H_{arom}), 7.38 – 7.31 (m, 4 H, H_{arom}), 7.16 (bt, 2 H, *J* = 7.3 Hz, H_{arom}), 5.57 (t, 1 H, *J* = 9.9 Hz, H-3), 5.47 (dd, 1 H, *J* = 10.2, 2.3 Hz, H-2), 4.90 (d, 1 H, *J* = 10.2 Hz, H-1), 4.89 (d, 1 H, *J* = 3.2 Hz, H-1′), 4.77 (dd, 1 H, *J* = 12.2, 3.3 Hz, H-6_a), 4.69 (dd, 1 H, *J* = 12.2, 7.6 Hz, H-6_b), 4.36 (d, 1 H *J* = 1.4 Hz, H-4), 4.31 (dd, 1 H, *J* = 12.6, 1.5 Hz, H-6_a′), 4.27 (d, 1 H, *J* = 2.3 Hz, H-4′), 4.20 (dd, 1 H, *J* = 7.8, 3.1 Hz, H-5), 4.11 – 4.05 (m, 2 H, H-2′ and H-6_b′), 3.94 (dd, 1 H, *J* = 9.7, 2.8 Hz, H-3′), 3.58 (bs, 1 H, H-5′), 1.06 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.97 (s, 18 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.83 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.21 (s, 3 H, $CH_{3\text{-}TBS}$), 0.18 (s, 3 H, $CH_{3\text{-}TBS}$), 0.05 (s, 3 H, $CH_{3\text{-}TBS}$), 0.00 (s, 3 H, $CH_{3\text{-}TBS}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.0, 165.9, 164.9 ($C{=}O_{Bz}$ x3), 133.8, 133.4, 133.14, 133.07 (CH_{arom} x4), 131.7 ($C_{q\text{-}arom}$), 130.1 (CH_{arom}), 130.0 ($C_{q\text{-}arom}$), 129.81, 129.73 (CH_{arom} x2), 129.4 ($C_{q\text{-}arom}$), 129.2 (CH_{arom}), 128.7 ($C_{q\text{-}arom}$), 128.5, 128.44, 128.37, 128.28, 127.9, 127.6 (CH_{arom} x6), 101.3 (C-1′), 85.6 (C-1), 77.5 (C-5), 76.1 (C-4), 74.9 (C-4′), 74.7 (C-2), 70.9 (C-3′), 70.3 (C-2′), 68.9 (C-5′), 67.7 (C-5), 67.1 (C-6′), 64.9 (C-6), 27.5, 27.3, 26.19, 26.18 ($CH_{3\text{-}tBu\text{-}Si}$ x4), 23.4, 20.7, 18.3, 18.2 ($C_{q\text{-}tBu\text{-}Si}$ x4), −3.9, −4.2, −4.3, −4.7 ($CH_{3\text{-}TBS}$ x4); IR (neat): 3070, 2932, 2857, 1729, 1270, 1093, 1069, 837, 709 cm^{-1}; HRMS Calcd. for $[C_{59}H_{82}O_{13}SSi_3 + Na]^+$: 1137.4676, found 1137.4676.

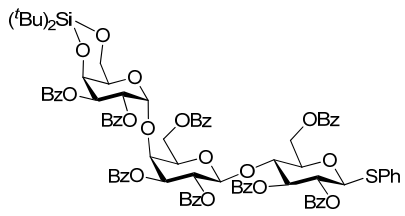


Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-*α*-D-galactopyranosyl)-1-thio-*β*-D-glucopyranoside (20) Method A: Galactose hydroxy donor **(12)** (64 mg, 0.12 mmol, 1.2 eq), diphenylsulfoxide (54 mg, 0.26 mmol, 2.6 eq) and 2,4,6-tris-*tert*-butylpyrimidine (30 mg, 0.12 mmol, 1.2 eq), were dissolved in anhydrous DCM (3 mL), under an atmosphere of argon. Activated molsieves (3Å) were added and the mixture was stirred for 1 hour at ambient temperature. The solution was then cooled to −60 °C and trifluoromethanesulfonic anhydride (22 µL, 0.13 mmol, 1.3 eq) was added. The mixture was warmed to −40 °C and stirred at this temperature for one hour. The reaction was then cooled to −60 °C and galactose acceptor **(7)** (59 mg, 0.10 mmol, 1.0 eq) was added as a solution in anhydrous DCM (2 mL). The reaction was stirred for three hours while reaching ambient temperature and was then quenched by addition of anhydrous triethylamine (0.14 mL, 1.0 mmol, 10 eq). The mixture was transferred to an extraction funnel using EtOAc (40 mL) and the organics were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtrated and concentrated *in vacuo*. Purification by column chromatography (5–20% EtOAc in petroleum ether) produced the title compound as a white solid (33 mg, 30 µmol, 30%). **Method B:** Galactoside imidate donor **(14)** (0.11 g, 0.15 mmol, 1.5 eq) and galactose acceptor **(7)** (59 mg, 0.10 mmol, 1.0 eq) were coevaporated twice in anhydrous toluene (4 mL) and then dissolved in anhydrous DCM (3 mL). The solution was cooled to 0°C and trifluoromethansulfonic acid (0.9 µL, 10 µmol, 0.1 eq) was added. The reaction was stirred for two hours at 0 °C and was then quenched by addition of triethylamine (0.14 ml, 1.0 mmol, 10 eq). The organics were transferred to an extraction funnel using EtOAc (40 mL) and the organics were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtrated and concentrated *in vacuo*. Purification by column chromatography (5–20% EtOAc in petroleum ether) produced the title compound as a white solid (82 mg, 75 µmol, 75%). R_f = 0.18 (15% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +51 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 – 8.05 (m, 2 H, H_{arom}), 8.05 – 8.02

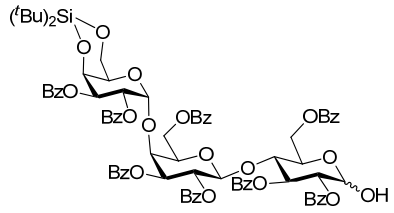
(m, 2 H, H_{arom}), 7.96 – 7.91 (m, 4 H, H_{arom}), 7.78 – 7.74 (m, 2 H, H_{arom}), 7.67 – 7.63 (m, 2 H, H_{arom}), 7.54 – 7.47 (m, 4 H, H_{arom}), 7.44 – 7.30 (m, 12 H, H_{arom}), 7.22 (bt, 2 H, *J* = 7.7 Hz, H_{arom}), 5.73 (dd, 1 H, *J* = 10.7, 3.6 Hz, H-2′), 5.51 (dd, 1 H, *J* = 10.7, 3.1 Hz, H-3′), 5.50 – 5.44 (m, 2 H, H-2 and H-3), 5.37 (d, 1 H, *J* = 3.6 Hz, H-1′), 4.89 (d, 1 H, *J* = 8.3 Hz, H-1), 4.86 (dd, 1 H *J* = 11.1, 6.3 Hz, H-6_a), 4.75 (d, 1 H, *J* = 3.1 Hz, H-4′), 4.42 (dd, 1 H, *J* = 11.1, 6.5 Hz, H-6_b), 4.26 (bs, 1 H, H-4), 4.21 (dd, 1 H, *J* = 13.1, 1.7 Hz, H-6_a′), 4.10 (t, 1 H, *J* = 6.5 Hz, H-5), 3.98 (dd, 1 H, *J* = 13.1, 2.2 Hz, H-6_b′), 3.41 (bs, 1 H, H-5′), 1.07 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.99 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.3, 166.0, 165.9, 165.6, 164.8 ($C{=}O_{Bz}$ x5), 135.6, 133.5, 133.3, 133.2, 133.04, 133.02, 130.2 (CH_{arom} x7), 123.0, 129.9 ($C_{q\text{-}arom}$ x2), 129.73, 129.70, 129.67, 129.6 (CH_{arom} x4), 129.4, 129.2, 128.8 ($C_{q\text{-}arom}$ x3), 128.70 (CH_{arom}), 128.67 ($C_{q\text{-}arom}$), 128.5, 128.39, 128.35, 128.34, 128.2 (CH_{arom} x5), 98.7 (C-1′), 84.8 (C-1), 76.7 (C-4), 76.3 (C-5), 74.4 (C-3), 71.1 (C-4′), 70.5 (C-2), 69.5 (C-2′), 67.9 (C-5′), 67.6 (C-3′), 66.7 (C-6′), 62.2 (C-6), 27.5, 27.2($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.3, 20.7 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 3072, 2935, 2858, 1718, 1269, 1093, 1070, 1027, 707 cm^{-1}; HRMS Calcd. for $[C_{61}H_{62}O_{15}SSi + Na]^+$: 1117.3471, found 1117.3477.



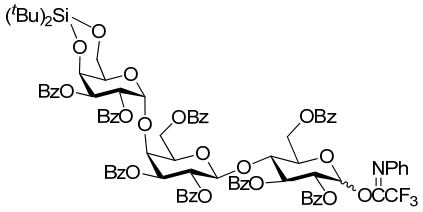
Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-bis-*O*-(*tert*-butyldimethylsilyl)-4,6-*O*-(di-tert-butylsilanediyl)-*α*-D-galacto-pyranosyl)-*β*-D-galactopyranosyl]-1-thio-*β*-D-glucopyranoside (23) Galactose hydroxyl donor **(10)** (66 mg, 0.12 mmol, 1.2 eq), diphenylsulfoxide (53 mg, 0.26 mmol, 2.6 eq) and 2,4,6-tris-*tert*-butylpyrimidine (75 mg, 0.30 mmol, 3.0 eq), were dissolved in anhydrous DCM (4 mL), under an atmosphere of argon. Activated molsieves (3Å) were added and the mixture was stirred for one hour at ambient temperature. The solution was then cooled to −60 °C and trifluoromethanesulfonic anhydride (22 µL, 0.13 mmol, 1.3 eq) was added. The mixture was warmed to −40 °C and stirred at this temperature for one hour. The reaction was then cooled to −60 °C and lactose acceptor **(18)** (0.11 g, 0.10 mmol, 1.0 eq) was added as a solution in anhydrous DCM (2 mL). The reaction was stirred for three hours while reaching ambient temperature and was then quenched by addition of anhydrous triethylamine (0.14 mL, 1.0 mmol, 10 eq). The mixture was transferred to an extraction funnel using EtOAc (40 mL) and the organics were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtrated and concentrated *in vacuo*. Purification by column chromatography (10–30% diethylether in petroleum ether) produced the title compound as a white solid (53 mg, 33 µmol, 33%). R_f = 0.28 (15% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 – 7.96 (m, 4 H, H_{arom}), 7.92 – 7.86 (m, 6 H, H_{arom}), 7.77 – 7.72 (m, 2 H, H_{arom}), 7.58 – 7.49 (m, 3 H, H_{arom}), 7.46 – 7.28 (m, 12 H, H_{arom}), 7.24 – 7.06 (m, 6 H, H_{arom}), 7.02 (m, 2 H, H_{arom}), 5.80 (t, 1 H, *J* = 9.2 Hz, H-3), 5.61 (dd, 1 H, *J* = 10.7, 8.0 Hz, H-2′), 5.32 (dd, 1 H, *J* = 10.7, 2.3 Hz, H-3′), 5.25 (t, 1 H, *J* = 9.5 Hz, H-2), 4.95 – 4.88 (m, 2 H, H-1′ and H-1″), 4.78 (d, 1 H, *J* = 9.9 Hz, H-1), 4.71 – 4.62 (m, 2 H, H-6_a and H-6_a′), 4.51 (dd, 1 H, *J* = 11.9, 5.5 Hz, H-6_b), 4.38 (d, 1 H, *J* = 11.4 Hz, H-6_a″), 4.33 (m, 1 H, H-4″), 4.29 – 4.21 (m, 2 H, H-4′ and H-6_b″), 4.19 – 4.11 (m, 3 H, H-6_a′ and H-4), 4.09 – 4.03 (m, 2 H, H-5″ and H-2″), 3.94 (m, 1 H, H-5), 3.85 (m, 2 H, H-5′ and H-3″), 1.03 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.97 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.90 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.73 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.04 (s, 3 H, $CH_{3\text{-}TBS}$), 0.03 (s, 3 H, $CH_{3\text{-}TBS}$), −0.05 (s, 3 H, $CH_{3\text{-}TBS}$), −0.07 (s, 3 H, $CH_{3\text{-}TBS}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.9, 165.8, 165.72, 165.65, 165.2, 165.0 ($C{=}O_{Bz}$ x6), 133.4, 133.3, 133.19, 133.16, 132.8 (CH_{arom} x5), 131.9 ($C_{q\text{-}arom}$), 129.84, 129.81, 129.64, 129.61, 129.59 (CH_{arom} x5), 129.53, 129.49, 129.2, 128.9 ($C_{q\text{-}arom}$ x4), 128.8 (CH_{arom}), 128.7 ($C_{q\text{-}arom}$), 128.6, 128.38, 128.36, 128.3, 128.0 (CH_{arom} x5), 101.2 (C-1″), 94.2 (C-1′), 85.7 (C-1), 77.0 (C-5), 76.3 (C-4), 75.1 (C-4′), 74.2 (C-4″), 74.1 (C-5′), 72.7 (C-3), 71.0 (C-3′), 70.4 (C-2), 69.7 (C-3″), 68.9 (C-5″), 67.7 (C-2′), 67.2 (C-2″), 66.7 (C-6″), 62.7 (C-6′), 61.8 (C-6), 27.4, 27.3, 26.22, 26.16 ($CH_{3\text{-}tBu\text{-}Si}$ x4), 23.4, 20.7, 18.2, 18.1 ($C_{q\text{-}tBu\text{-}Si}$ x4), −4.0, −4.2, −4.5, −4.6 ($CH_{3\text{-}TBS}$ x4).



Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-*α*-D-galactopyranosyl)-*β*-D-galactopyranosyl]-1-thio-*β*-D-glucopyranoside (24) **Method A:** Galactose hydroxy donor **(12)** (63 mg, 0.12 mmol, 1.2 eq), diphenylsulfoxide (53 mg, 0.26 mmol, 2.6 eq) and 2,4,6-tris-*tert*-butylpyrimidine (30 mg, 0.12 mmol, 1.2 eq), were dissolved in anhydrous DCM (3 mL), under an atmosphere of argon. Activated molsieves (3Å) were added and the mixture was stirred for one hour at ambient temperature. The solution was then cooled to −60 °C and trifluoromethanesulfonic anhydride (22 µL, 0.13 mmol, 1.3 eq) was added. The mixture was warmed to −40 °C and stirred at this temperature for one hour. The reaction was then cooled to −60 °C and lactose acceptor **(18)** (0.11 g, 0.10 mmol, 1.0 eq) was added as a solution in anhydrous DCM (3 mL). The reaction was stirred for three hours while reaching ambient temperature and was then quenched by addition of anhydrous triethylamine (0.14 mL, 1.0 mmol, 10 eq). The mixture was transferred to an extraction funnel using EtOAc (40 mL) and the organics were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtrated and concentrated *in vacuo*. Purification by column chromatography (2–8% EtOAc, 20% DCM in petroleum ether) produced the title compound as a white solid (32 mg, 20 µmol, 20%). Method **B:** Imidate donor **(14)** (1.8 g, 2.6 mmol, 1.5 eq) and lactose acceptor **(18)** (1.8 g, 1.7 mmol, 1.0 eq) were coevaporated together two times in toluene (10 mL) and then dissolved in anhydrous DCM (17 mL) and cooled to 0 °C followed by addition of a catalytic amount of triflic acid (15 µL, 0.17 mmol, 0.1 eq). The reaction was stirred until TLC showed complete conversion of the lactose acceptor (~4 hours). The reaction mixture was then transferred to an extraction funnel with EtOAc (30 mL) and washed with sat. aq. $NaHCO_3$ (50 mL) and brine (40 mL). The aqueous layers were then extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by size exclusion column, followed by column chromatography (2–8% EtOAc, 20% DCM in petroleum ether) produced the title compound as a white solid (2.5 g, 1.6 mmol, 92%). R_f = 0.41 (20% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +49 (C = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.14 (dm, 2 H, *J* = 7.9 Hz, H_{arom}), 8.05 (dm, 2 H, *J* = 8.0 Hz, H_{arom}), 8.01 (dm, 2 H, *J* = 7.9 Hz, H_{arom}), 7.95 (dm, 2 H, *J* = 8.0 Hz, H_{arom}), 7.90 (dm, 2 H, *J* = 7.9 Hz, H_{arom}), 7.88 (dm, 2 H, *J* = 8.0 Hz, H_{arom}), 7.68 (dm, 2 H, *J* = 7.9 Hz, H_{arom}), 7.58 – 7.51 (m, 4 H, H_{arom}), 7.50 – 7.43 (m, 4 H, H_{arom}), 7.42 – 7.16 (m, 19 H, H_{arom}), 7.14 – 7.05 (m, 4 H, H_{arom}), 5.78 (t, 1 H, *J* = 9.2 Hz, H-3), 5.72 (dd, 1 H, *J* = 10.7, 3.7 Hz, H-2″), 5.61 (dd, 1 H, *J* = 10.8, 7.8 Hz, H-2′), 5.50 (dd, 1 H, *J* = 10.7, 3.1 Hz, H-3″), 5.39 (t, 1 H, *J* = 9.7 Hz, H-2), 5.36 (d, 1 H, *J* = 3.6 Hz, H-1″), 5.27 (dd, 1 H, *J* = 10.7, 2.1 Hz, H-3′), 5.09 (d, 1 H, *J* = 3.0 Hz, H-4″), 4.89 (d, 1 H, *J* = 10.0 Hz, H-1), 4.80 (d, 1 H, *J* = 7.8 Hz, H-1′), 4.59 (dd, 1 H, *J* = 12.1, 2.1 Hz, H-6_a), 4.52 (dd, 1 H, *J* = 12.9, 1.8 Hz, H-6_a″), 4.48 – 4.41 (m, 2 H, H-6_b and H-6_b″), 4.36 (bs, 1 H, H-5″), 4.11 – 4.05 (m, 2 H, H-4 and H-4′), 3.95 (dd, 1 H, *J* = 10.9, 5.5 Hz, H-6_a′), 3.87 (ddd, 1 H, *J* = 10.0, 5.4, 2.0 Hz, H-5), 3.76 (dd, 1 H, *J* = 10.9, 7.7 Hz, H-6_b′), 3.57 (dd, 1 H, *J* = 7.7, 5.5 Hz, H-5′), 1.06 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.99 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.2, 166.0, 165.8, 165.7, 165.14, 165.05, 164.83, 164.77 ($C{=}O_{Bz}$ x8), 133.4, 133.2, 133.16, 133.13, 133.08, 133.0, 132.9 (CH_{arom} x7), 131.7 ($C_{q\text{-}arom}$), 130.1, 129.98 (CH_{arom} x2), 129.94 ($C_{q\text{-}arom}$), 129.8, 129.66, 129.62 (CH_{arom} x3), 129.60, 129.56 ($C_{q\text{-}arom}$ x2), 129.52, 129.4 (CH_{arom} x2), 129.2, 129.0 ($C_{q\text{-}arom}$ x2), 128.7 (CH_{arom}), 128.64, 128.56, 128.52 ($C_{q\text{-}arom}$ x3), 128.46, 128.43, 128.36, 128.32, 128.18, 128.09 (CH_{arom} x6), 101.5 (C-1′), 98.7 (C-1″), 85.7 (C-1), 76.95 (C-5), 76.84 (C-4), 76.3 (C-4′), 74.2 (C-3), 72.78 (C-3′), 72.72 (C-5′), 71.2 (C-3″), 71.0 (C-4″), 70.4 (C-2), 69.7 (C-2′), 69.6 (C-2″), 68.3 (C-5″), 66.9 (C-6″), 62.6 (C-6), 60.5 (C-6′), 27.5, 27.2 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2, 20.7 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 3070, 2934, 2860, 1722, 1266, 1069, 706 cm^{-1}; HRMS Calcd. for $[C_{88}H_{84}O_{23}SSi + Na]^+$: 1591.4786, found 1591.4795.

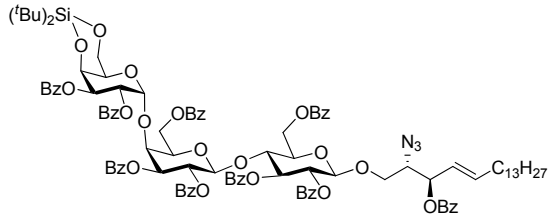


2,3,6-Tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-α-D-galactopyranosyl)-α-D-galactopyranosyl]-α/β-D-glucopyranose (31)
Phenyl thioglycotrioside **(24)** (2.2 g, 1.4 mmol, 1.0 eq) was dissolved in DCM (30 mL) and cooled to 0 °C. *N*-iodosuccinimide (0.35 g, 1.6 mmol, 1.1 eq) was added, before addition of trifluoroacetic acid (0.12 mL, 1.6 mmol, 1.1 eq). The reaction was left stirring under exposure to the atmosphere until TLC showed full conversion (~3 hours). The mixture was then transferred to an extraction funnel with EtOAc (50 mL) and washed with sodium thiosulfate (20% aq.) (70 mL), sat. aq. $NaHCO_3$ (70 mL) and brine (60 mL). The aqueous layers were extracted with EtOAc (70 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (0–10% acetone, 40% DCM in petroleum ether) afforded the title compound as a white solid (1.8 g, 1.2 mmol, 86%). R_f = 0.55 and 0.60 (20% acetone in petroleum ether); NMR assignment for major isomer (α): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (dm, 2 H, *J* = 7.6 Hz, H_{arom}), 8.10 – 8.03 (m, 4 H, H_{arom}), 8.02 – 7.88 (m, 6 H, H_{arom}), 7.76 – 7.70 (m, 2 H, H_{arom}), 7.62 – 7.26 (m, 22 H, H_{arom}), 7.26 – 7.19 (m, 2 H, H_{arom}), 7.16 (tm, 2 H, *J* = 7.8 Hz, H_{arom}), 6.17 (t, 1 H, *J* = 9.6 Hz, H-3), 5.75 (dd, 1 H, *J* = 10.6, 3.5 Hz, H-2″), 5.68 (m, 1 H, H-2′), 5.63 (d, 1 H, *J* = 3.5 Hz, H-1), 5.55 (dd, 1 H, *J* = 11.0, 3.3 Hz, H-3″), 5.41 (d, 1 H, *J* = 3.5 Hz, H-1″), 5.32 (d, 1 H, *J* = 10.1 Hz, H-3′), 5.23 (dd, 1 H, *J* = 10.2, 3.5 Hz, H-2), 5.11 (m, 1 H, H-4″), 4.90 (d, 1 H, *J* = 7.8 Hz, H-1′), 4.60 – 4.30 (m, 6 H, H-6_a, H-6_b, H-6_a″, H-6_b″, H-5 and H-5″), 4.23 – 4.07 (m, 3 H, H-4, H-4′ and H-6_a′), 4.87 (m, 1 H, H-6_b′), 3.58 (m, 1 H, H-5′), 1.09 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.03 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.3, 166.1, 165.99, 165.98, 165.8, 165.1, 164.9 (x2) ($C{=}O_{Bz}$ x8), 133.5, 133.4, 133.2, 133.1, 133.01, 132.96, 130.1, 129.9, 129.68, 129.66, 129.5, 129.4 (CH_{arom} x12), 129.1, 129.0, 128.9, 128.7, 128.6 ($C_{q\text{-}arom}$ x5), 128.49, 128.45, 128.40, 128.37, 128.3, 128.1 (CH_{arom} x6), 101.5 (C-1′), 98.7 (C-1″), 90.2 (C-1), 76.9 (C-4), 76.1 (C-4′), 73.0 (C-3′), 72.7 (C-5′), 72.2 (C-2), 71.2 (C-3″), 71.1 (C-4″), 70.3 (C-3), 69.9 (C-2″), 69.6 (C-2′), 68.4 (C-5″), 68.3 (C-5), 66.9 (C-6″), 62.3 (C-6), 60.5 (C-6′), 27.6 27.3 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.3, 20.8 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 3070, 2934, 2855, 1722, 1452, 1266, 1093, 1069, 1027, 706 cm^{-1}; HRMS Calcd. for $[C_{82}H_{80}O_{24}Si + Na]^+$: 1499.4701, found 1499.4707.



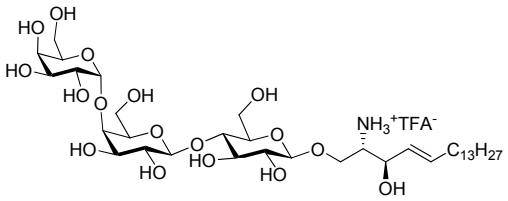
2,3,6-Tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-α-D-galactopyranosyl)-α-D-galactopyranosyl]-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)-β-D-glucopyranoside (32)
Protected globotriaosyl hemiacetal **(31)** (1.9 g, 1.3 mmol, 1.0 eq) was dissolved in acetone (30 mL) and cooled to 0 °C. Cesium carbonate (0.63 g, 1.9 mmol, 1.5 eq) was added followed by chloro *N*-phenyl-trifluoroimidate (0.40 g, 1.9 mmol, 1.5 eq) and the reaction was stirred at 0 °C for two hours. The reaction mixture was filtrated and concentrated *in vacuo*. Purification by column chromatography, which initially was neutralized by running an eluent of 3% Et_3N in petroleum ether (200 mL) through the column, (0–10% EtOAc, 20% DCM in petroleum ether) produced the title compound as a white solid (1.9 g, 1.2 mmol, 92%). R_f = 0.47 and 0.42 (10% EtOAc and 20% DCM in petroleum ether); NMR assignment of major isomer ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (dm, 2 H, *J* = 7.2 Hz, H_{arom}), 8.08 – 8.02 (m, 4 H, H_{arom}), 7.98 (dm, 2 H, *J* = 7.5 Hz, H_{arom}), 7.93 (dm, 4 H, *J* = 7.9 Hz, H_{arom}), 7.69 (dm, 2 H, *J* = 7.8 Hz, H_{arom}), 7.57 – 7.51 (m, 4 H, H_{arom}), 7.50 – 7.43 (m, 4 H, H_{arom}), 7.42 – 7.25 (m, 16 H, H_{arom}), 7.12 (tm, 2 H, *J* = 7.8 Hz, H_{arom}), 7.05 (tm, 2 H, *J* = 7.8 Hz, $H_{arom\text{-}NPh}$), 6.94 (tm, 1 H, *J* = 7.4 Hz, $H_{arom\text{-}NPh}$), 6.71 (bs, 1 H, C-1), 6.36 (bs, 2 H, $H_{arom\text{-}NPh}$), 6.14 (t, 1 H, *J* = 9.2 Hz, H-3), 5.75 (dd, 1 H, *J* = 10.7, 3.6 Hz, H-2″), 5.69 (dd, 1 H,

J = 10.8, 7.8 Hz, H-2′), 5.54 (dd, 1 H, *J* = 10.7, 3.0 Hz, H-3′′), 5.49 (m, 1 H, H-2), 5.40 (d, 1 H, *J* = 3.6 Hz, H-1′′), 5.30 (dd, 1 H, *J*= 10.9, 2.1 Hz, H-3′), 5.11 (d, 1 H, *J* = 3.1 Hz, H-4′′), 4.90 (d, 1 H, *J* = 7.8 Hz, H-1′), 4.64 – 4.35 (m, 5 H, H-6_a, H-6_b, H-6_a′′, H-6_b′′ and H-5′′), 4.31 – 4.21 (m, 2 H, H-4, H-5), 4.15 – 4.09 (m, 1 H, H-4′), 4.05 (dd, 1 H, *J* = 10.9, 5.1 Hz, H-6_a′), 3.81 (m, 1 H, H-6_b′), 3.55 (dd, 1 H, *J* = 8.0, 5.4 Hz, H-5′), 1.07 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.01 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.2, 166.0, 165.7, 165.6, 165.4, 164.80, 164.77, 164.7 ($C{=}O_{Bz}$ x8), 142.8 (C=N), 133.6, 133.4, 133.2, 133.14, 133.10, 133.06, 132.95, 132.87, 130.1, 129.94 (CH_{arom} x10), 129.91 ($C_{q\text{-}arom}$), 129.89, 129.63, 129.59, 129.57, 129.5 (CH_{arom} x5), 129.4 ($C_{q\text{-}arom}$), 129.3 (CH_{arom}), 129.0, 128.6 ($C_{q\text{-}arom}$ x2), 128.53, 128.50 (CH_{arom} x2), 128.48 ($C_{q\text{-}arom}$), 128.44, 128.40, 128.36, 128.35, 128.3, 128.1, 128.0 (CH_{arom} x7), 124.2 (C-1), 119.0 ($CH_{arom\text{-}NPh}$), 115.8 (q, *J* = 285.7 Hz, CF_3), 101.8 (C-1′), 98.8 (C-1′′), 76.4 (C-4), 76.1 (C-4′), 72.9 (C-3′), 72.7 (C-5′′), 71.3 (C-3′′), 71.2 (C-5), 71.0 (C-4′′), 70.2 (C-2 and C-3), 69.8 (C-2′), 69.5 (C-2′′), 68.3 (C-5′), 66.9 (C-6′′), 61.7 (C-6), 60.1 (C-6′), 27.5, 27.2 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2, 20.7 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 3069, 2936, 2860, 1718, 1452, 1266, 1093, 1070, 907, 730, 705 cm^{-1}; HRMS Calcd. for $[C_{90}H_{84}F_3NO_{24}Si + Na]^+$: 1670.4997, found 1670.5009.



2-Azido-3-*O*-benzoyl-D-*erythro*-sphingosine-1-yl 2,3,6-tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-(di-tert-butylsilanediyl)-*α*-D-galactopyranosyl)-*α*-D-galactopyranosyl]-*β*-D-glucopyranoside (33) Globotriaosylimidate donor **(32)** (18 mg, 0.11 mmol, 1.2 eq) and sphingosine acceptor **(30)** (39 mg, 91 µmol, 1.0 eq) were coevaporated twice in toluene (5 mL) and then dissolved in anhydrous DCM (3 mL). Activated molsieves (3Å) were added and the mixture was stirred for 1 hour at ambient temperature and then cooled to 0 °C, before addition of a catalytic amount of triflic acid (0.8 µL, 9.1 µmol, 0.1 eq). The reaction was stirred at 0 °C until TLC showed complete conversion of the sphingosine acceptor (~2 hours). The reaction was quenched by addition of triethylamine (0.13 mL, 0.91 mmol, 10 eq). The organics were then transferred to an extraction funnel with EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (30 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (10–20% EtOAc and 10% DCM in petroleum ether) produced the title compound as an amorphous solid (138 mg, 73 µmol, 80%). R_f = 0.35 (20% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +29 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17 (dm, 2 H, *J* = 7.2 Hz, H_{arom}), 8.07 – 7.99 (m, 4 H, H_{arom}), 7.97 (dm, 4 H, *J* = 7.6 Hz, H_{arom}), 7.89 (dm, 4 H, *J* = 7.4 Hz, H_{arom}), 7.69 (dm, 2 H, *J* = 7.2 Hz, H_{arom}), 7.57 (dm, 2 H, *J* = 7.2 Hz, H_{arom}), 7.54 – 7.42 (m, 8 H, H_{arom}), 7.41 – 7.27 (m, 15 H, H_{arom}), 7.21 (tm, 2 H, *J* = 7.6 Hz, , H_{arom}), 7.11 (tm, 2 H, *J* = 7.7 Hz, H_{arom}), 5.78 (t, 1 H, *J* = 9.1 Hz, H-3), 5.73 (dd, 1 H, *J* = 10.7, 3.6 Hz, H-2′′), 5.67 (m, 1 H, H-5_{Sp}), 5.62 (dd, 1 H, *J* = 11.2, 7.7 Hz, H-2′), 5.55 – 5.48 (m, 2 H, H-3′′ and H-3_{Sp}), 5.47 – 5.38 (m, 2 H, H-2 and H-4_{Sp}), 5.38 (d, 1 H, *J* = 3.4 Hz, H-1′′), 5.28 (dd, 1 H, *J* = 10.9, 2.1 Hz, H-3′), 5.10 (d, 1 H, *J* = 2.8 Hz, H-4′′), 4.83 (d, 1 H, *J* = 7.8 Hz, H-1′), 4.71 (d, 1 H, *J* = 7.6 Hz, H-1), 4.57 – 4.48 (m, 2 H, H-6_a and H6_a′′), 4.46 – 4.39 (m, 2 H, H-6_b and H6_b′′), 4.37 (bs, 1 H, H-5′′), 4.21 (t, 1 H, *J* = 9.4 Hz, H-4), 4.09 (bs, 1 H, H-4′), 3.98 (dd, 1 H, *J* = 10.9, 5.4 Hz, H-6_a′), 3.91 – 3.77 (m, 4 H, H-$1_{a\text{-}Sp}$, H-2_{Sp}, H-5 and H-6_b′), 3.58 – 3.48 (m, 2 H, H-5′ and H-$1_{b\text{-}Sp}$), 1.87 (m, 2 H, H-6_{Sp}), 1.33 – 1.12 (m, 22 H, H-7_{Sp} to H-17_{Sp}), 1.06 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.00 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18_{Sp}); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.2, 166.0, 165.8, 165.7, 165.1, 165.0, 164.9, 164.81, 164.78 ($C{=}O_{Bz}$ x9), 138.9 (C-5_{Sp}), 133.4, 133.2, 133.1, 133.04, 132.98, 132.9, 130.1, 130.0 (CH_{arom} x8), 129.93, 129.89 ($C_{q\text{-}arom}$ x2), 129.8, 129.7, 129.63, 129.56, 129.51 (CH_{arom} x5), 129.46

($C_{q\text{-arom}}$), 129.4 (CH_{arom}), 129.3, 129.1, 128.6, 128.53, 128.50 ($C_{q\text{-arom}}$ x5), 128.48, 128.4, 128.3, 128.2, 128.1 (CH_{arom} x5), 122.3 (C-4_{Sp}), 101.4 (C-1′), 100.6 (C-1), 98.7 (C-1″), 76.7 (C-4), 76.2 (C-4′), 74.8 (C-3_{Sp}), 73.1 (C-3), 73.0 (C-5), 72.8 (C-3′), 72.7 (C-5′), 71.7 (C-2), 71.2 (C-3″), 71.0 (C-4″), 69.7 (C-2′), 69.5 (C-2″), 68.6 (C-5″), 68.1 (C-1_{Sp}), 66.9 (C-6″), 63.4 (C-2_{Sp}), 62.2 (C-6), 60.5 (C-6′), 32.2 (C-6_{Sp}), 31.9, 29.64, 29.61 (x2), 29.59, 29.5, 29.31, 29.30, 29.1, 28.5 ($CH_{2\text{-Sp}}$ x10), 27.5, 27.2 ($CH_{3\text{-tBu-Si}}$ x2), 23.2 ($C_{q\text{-tBu-Si}}$), 22.7 ($CH_{2\text{-Sp}}$), 20.7 ($C_{q\text{-tBu-Si}}$), 14.1 (C-18_{Sp}); IR (neat): 3070, 2926, 2856, 1722, 1451, 1265, 1093, 1069, 909, 704 cm^{-1}; HRMS Calcd. for $[C_{107}H_{117}N_3O_{26}Si + Na]^+$: 1910.7587, found 1910.7584.



Globotriaosylsphingosine (34) Protected globotriaosylsphingosine (**33**) (70 mg, 40 µmol, 1.0 eq) was dissolved in DCM:MeOH 1:4 (5 mL) and sodium methoxide (30% in MeOH) (5 µL, 40 µmol, 1.0 eq) was added. The reaction was stirred over night at ambient temperature and the progression of the reaction was followed by analytical HPLC-MS. The reaction was quenched with Amberlite H^+, filtered and concentrated *in vacuo*. The crude reaction mixture was then dissolved in pyridine (4 mL) and HF·pyridine (8.0 µL, 8.0 eq based on HF) was added. The reaction was again run over night at ambient temperature followed by concentration *in vacuo*. The residue was roughly purified over a short silica column and eluted with MeOH/$CHCl_3$ 1:3, afterwhich the residue was dissolved in a pyridine:Et_3N:MeOH mixture 2:1:1 (8 mL) and purged with H_2S at 0 °C for 30 minutes. The reaction was stirred at ambient temperature over night and the completion of the reaction was monitored by analytical HPLC-MS. The reaction mixture was purged with argon gas and was then concentrated *in vacuo*. Purification by HPLC-MS (24–46% B, 3 CV, following the general procedure for HPLC-MS purifications) produced the title compound (TFA-salt) as a white powder after lyophilization (24 mg, 27 µmol, 72%). $[\alpha]_D^{22}$: +32 (C = 0.1 MeOH); ^{1}H NMR (600 MHz, MeOD-d_4) δ: 5.87 (dtd, 1 H, *J* = 15.4, 6.8, 1.3 Hz, H-5_{Sp}), 5.49 (ddt, 1 H, *J* = 15.4, 6.8, 1.5 Hz, H-4_{Sp}), 4.94 (d, 1 H, *J* = 3.9 Hz, H-1″), 4.40 (d, 1 H, *J* = 6.9 Hz, H-1), 4.37 (d, 1 H, *J* = 7.8 Hz, H-1′), 4.32 (ddd, 1 H, *J* = 6.8, 4.7, 1.3 Hz, H-3_{Sp}), 4.25 (ddd, 1 H, *J* = 7.1, 5.2, 1.3 Hz, H-5″), 4.01 – 3.96 (m, 2 H, H-4′and H-$1_{a\text{-Sp}}$), 3.94 (dd, 1 H, *J* = 11.9, 2.6 Hz, H-6_a), 3.93 – 3.91 (m, 2 H, H-4″ and H-$1_{b\text{-Sp}}$), 3.89 (dd, 1 H, *J* = 7.7, 4.1 Hz, H-6_b), 3.88 – 3.81 (m, 3 H, H-6_a′, H-6_b′ and H-2″), 3.77 (dd, 1 H, *J* = 10.2, 3.2 Hz, H-3″), 3.74 (dd, 1 H, *J* =11.1, 7.1 Hz, H-6_a″), 3.71 – 3.66 (m, 2 H, H-5′ and H-6_b″), 3.58 – 3.51 (m, 4 H, H-4, H-3, H-3′ and H-2′), 3.47 (m, 1 H, H-5), 3.40 (ddd, 1 H, *J* = 8.5, 4.7, 3.6 Hz, H-2_{Sp}), 3.30 (t, 1 H, *J* = 7.7 Hz, H-2), 2.10 (q, 2 H, *J* = 7.0 Hz, H-6_{Sp}), 1.42 (m, 2 H, H-7_{Sp}), 1.36 – 1.25 (m, 20 H, H-8_{Sp} to H-17_{Sp}), 0.90 (t, 3 H, *J* = 7.0 Hz, H-18_{Sp}); ^{13}C NMR (151 MHz, MeOD-d_4) δ: 136.8 (C-5_{Sp}), 128.3 (C-4_{Sp}), 105.4 (C-1′), 103.7 (C-1), 102.7 (C-1″), 80.8 (C-4), 79.8 (C-4′), 76.6 (C-5′ and C-5), 76.3 (C-2′), 74.7 (C-3), 74.6 (C-2), 72.8 (C-5″), 72.6 (C-3′), 71.3 (C-4″), 71.0 (C-3″), 70.8 (C-3_{Sp}), 70.5 (C-2″), 67.1 (C-1_{Sp}), 62.7 (C-6″), 61.6 (C-6), 61.5 (C-6′), 56.7 (C-2_{Sp}), 33.4 (C-6_{Sp}), 33.1, 30.79 (x3), 30.76, 30.74, 30.6, 30.5, 30.4, 30.2, 23.7 ($CH_{2\text{-Sp}}$ x11), 14.4 (C-18_{Sp}); IR (neat): 3344 bs, 2925, 2855, 1674, 1202, 1134, 1067, 1027, 974, 801, 721 cm^{-1}; HRMS Calcd for $[C_{36}H_{67}NO_{17} + H]^+$: 786.4482 Found 786.4485.

References and footnotes

1. Kihlberg, J.; Magnusson, G. *Pure Appl. Chem.* **1996,** *68* (11), 2119-2128.
2. Sharon, N. *Biochim. Biophys. Acta* **2006,** *1760* (4), 527-537.
3. Hakomori, S.-I. *Chem. Phys. Lipids* **1986,** *42* (1-3), 209-233.
4. Nudelman, E.; Kannagi, R.; Hakomori, S.; Parsons, M.; Lipinski, M.; Wiels, J.; Fellous, M.; Tursz, T. *Science* **1983,** *220* (4596), 509-511.
5. Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999,** *38* (11), 1532-1568.

6. Gieselmann, V. *Biochim. Biophys. Acta* **1995,** *1270* (2-3), 103-136.
7. Kint, J. A. *Science* **1970,** *167* (3922), 1268-1269.
8. Aerts, J. M.; Groener, J. E.; Kuiper, S.; Donker-Koopman, W. E.; Strijland, A.; Ottenhoff, R.; van Roomen, C.; Mirzaian, M.; Wijburg, F. A.; Linthorst, G. E.; Vedder, A. C.; Rombach, S. M.; Cox-Brinkman, J.; Somerharju, P.; Boot, R. G.; Hollak, C. E.; Brady, R. O.; Poorthuis, B. J. *Proc. Natl. Acad. Sci. U. S. A.* **2008,** *105* (8), 2812-2817.
9. Togawa, T.; Kodama, T.; Suzuki, T.; Sugawara, K.; Tsukimura, T.; Ohashi, T.; Ishige, N.; Suzuki, K.; Kitagawa, T.; Sakuraba, H. *Mol. Genet. Metab. 100* (3), 257-261.
10. Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998,** (8), 1075-1091.
11. Cox, D. D.; Metzner, E. K.; Reist, E. J. *Carbohydr. Res.* **1978,** *63*, 139-147.
12. Shapiro, D.; Acher, A. J. *Chem. Phys. Lipids* **1978,** *22* (3), 197-206.
13. Dahmen, J.; Frejd, T.; Magnusson, G.; Noori, G.; Carlstrom, A. S. *Carbohydr. Res.* **1984,** *127* (1), 15-25.
14. Wang, C. N.; Li, Q.; Wang, H. S.; Zhang, L. H.; Ye, X. S. *Tetrahedron* **2006,** *62* (50), 11657-11662.
15. Bhattacharyya, S.; Magnusson, B. G.; Wellmar, U.; Nilsson, U. J. *J. Chem. Soc., Perkin Trans. 1* **2001,** (8), 886-890.
16. Miura, T.; Inazu, T. *Tetrahedron Lett.* **2003,** *44* (9), 1819-1821.
17. Hada, N.; Oka, J.; Nishiyama, A.; Takeda, T. *Tetrahedron Lett.* **2006,** *47* (37), 6647-6650.
18. Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005,** *46* (11), 1873-1875.
19. Deng, S.; Yu, B.; Guo, Z.; Hui, Y. *J. Carbohydr. Chem.* **1998,** *17* (3), 439 - 452.
20. Khiar, N.; Martin-Lomas, M. *J. Org. Chem.* **1995,** *60* (21), 7017-7021.
21. Corey, E. J.; Hopkins, P. B. *Tetrahedron Lett.* **1982,** *23* (47), 4871-4874.
22. Codée, J. D. C.; van den Bos, L. J.; Litjens, R.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. H.; van der Marel, G. A. *Tetrahedron* **2004,** *60* (5), 1057-1064.
23. Garcia, B. A.; Poole, J. L.; Gin, D. Y. *J. Am. Chem. Soc.* **1997,** *119* (32), 7597-7598.
24. Dinkelaar, J.; Witte, M. D.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Carbohydr. Res.* **2006,** *341* (10), 1723-1729.
25. Motawia, M. S.; Marcussen, J.; Møller, B. L. *J. Carbohydr. Chem.* **1995,** *14* (9), 1279 - 1294.
26. Yu, B.; Tao, H. *Tetrahedron Lett.* **2001,** *42* (12), 2405-2407.
27. Muller, D.; Vic, G.; Critchley, P.; Crout, D. H. G.; Lea, N.; Roberts, L.; Lord, J. M. *J. Chem. Soc., Perkin Trans. 1* **1998,** (15), 2287-2294.
28. Nagao, Y.; Nekado, T.; Ikeda, K.; Achiwa, K. *Chem. Pharm. Bull.* **1995,** *43* (9), 1536-1542.
29. Kim, S.; Chang, H.; Kim, W. J. *J. Org. Chem.* **1985,** *50* (10), 1751-1752.
30. See chapter 3 for additional information.
31. Kim, S.; Lee, S.; Lee, T.; Ko, H.; Kim, D. *J. Org. Chem.* **2006,** *71* (22), 8661-8664.
32. Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007,** *9* (19), 3797-3800.
33. Duclos, R. I. *Chem. Phys. Lipids* **2001,** *111* (2), 111-138.
34. Yao, Q. J.; Song, J.; Xia, C. F.; Zhang, W. P.; Wang, P. G. *Org. Lett.* **2006,** *8* (5), 911-914.
35. Since there are no functionalities present in the product that can be detected with spectroscopic methods, the elution of the products were monitored by MS using a splitting device.
36. Gold, H.; Boot, R. G.; Aerts, J. M. F. G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *Eur. J. Org. Chem.* **2011,** (9), 1652-1663.

Chapter 3

Synthesis of $^{13}C_5$-Labeled Sphingosine and $^{13}C_5$-Globotriaosylsphingosine

Introduction

Metabolic disorders belong to a group of pathological conditions in which enzymes involved in metabolic pathways (i.e. anabolism and catabolism) are either dysfunctional or absent. The ability to follow specific metabolic pathways constitutes a key to the understanding of these pathological processes. The pioneering attempts to follow a metabolic pathway were made by Dakin as early as in 1908.[1] Dakin investigated the *in vivo* oxidation of fatty acids by introduction of an "inert" phenyl tag that made it possible to distinguish the artificial from the endogenous substrates. To prevent biases such as different metabolic handling between probes and endogenous substrates, probes are needed that as far as possible imitate the physiochemical properties of the natural substrates. Following the discovery and isolation of deuterium in 1932,[2] Schoenheimer was the first to show how substrates labeled with isotopes (isotopomeres) could fulfil these requirements in a study of fatty acid and sterol metabolism.[3] In his pioneering work, Schoenheimer also studied protein metabolism by the incorporation of a ^{15}N-isotope in tyrosine.[4] This methodology – commonly referred to as the isotopic labeling methodology – has evolved since then and includes both incorporation of stable isotopes (^{2}H, ^{13}C, ^{15}N, ^{18}O, ^{34}S *etc.*) and radionuclides (^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{125}I *etc.*). For example, uniformly ^{13}C-labeled D-glucose ([U-^{13}C]-D-glucose) has been used to study the metabolism of nutrients.[5] The isotopes of [U-^{13}C]-D-glucose are distributed throughout the metabolic network and its

metabolites can be followed and analyzed by means of ^{13}C-mass isotopomer distribution (MID). The development of efficient separation techniques and the accuracy of HRMS are of great importance for these experiments. Furthermore, NMR techniques are routinely used for the investigation of isotopically labeled substrates. For example, protein-protein interactions can be investigated by chemical-shift perturbation experiments using ^{15}N-enriched proteins.[6] NMR can also be employed to study enzyme mechanisms/interactions by monitoring the effect of the binding of ^{13}C-enriched substrates or cofactors on proteins.[7] Chemical synthesis does not only allow a site-directed introduction of isotopic labels, but uniformly labeled entities as complex as [U-^{13}C]-retinal have also been realized.[8]

Fabry's disease belongs to a family of metabolic disorders, commonly referred to as lysosomal storage disorders, as already mentioned in **Chapter 2**. This disease is characterized by an impaired activity of *α*-galactosidase A, an enzyme involved in the catabolism of glycosphingolipids, which results in accumulation of its substrate globotriaosylceramide (CTH) in the lysosomes of epithelial cells.[9] Interestingly, it is the concentration of globotriaosylsphingosine (lysoCTH), the deacylated form of CTH in blood, which seems to have the best correlation with the disease manifestations in Fabry patients.[10] To continue the efforts already made in the field of glycosphingolipid metabolism and its involvement in lysosomal storage disorders, utilization of the isotopic labeling methodology would provide additional information. In this context, site-directed introduction of stable isotopes into the D-*erythro*-sphingosine part of (glyco)sphingolipids would provide a tool that can function as an internal standard for the quantification of numerous endogenous (glyco)sphingolipids in man. The labeled structures can also be utilised as isotopic tracers to follow specific metabolic pathways in pathological sphingolipidoses.[11] Previously, fluorescent sphingolipid probes have been used in the study of sphingolipid metabolism[12] and it has also been reported that BODIPY-labeled glycosphingolipids do not give any adverse effect of the endocytosis of the probes.[13] However, alteration of the trafficking and compartmentalization of these sphingolipid analogues, due to different physiochemical properties as compared to the endogenous compounds, cannot be excluded.

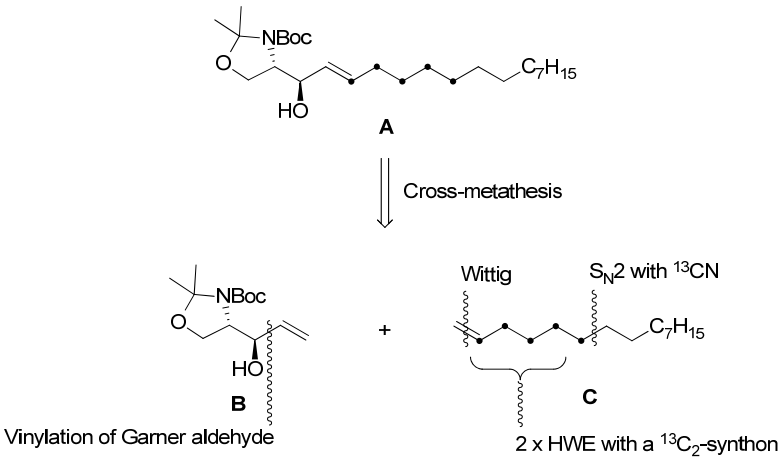
The current chapter describes the synthesis of a $^{13}C_5$-labeled D-*erythro*-sphingosine and its subsequent use in the construction of a [$^{13}C_5$]-lysoCTH. In the synthesis of the isotopically labeled D-*erythro*-sphingosine the following aspects have been taken into account. First, to minimize the possibility of undesired isotopic effects incorporation of ^{13}C-atoms as isotopic labels are preferred. It has been shown that deuterated substrate analogues might possess significantly different physiochemical properties as compared to their unlabeled counterparts.[14] Second, introduction of five labels appears optimal since it would provide a standard with a clear mass difference compared to both the endogenous substrate and homologues thereof. Third, a good ^{13}C-atom economy is important to enable a cost-efficient synthesis of the product. Robust and efficient chemistry using ^{13}C-labeled reagents, derived from relatively inexpensive commercial sources are desired to fulfill these requirements.

Results and Discussion

Due to its biological importance, numerous attempts to synthesize D-*erythro*-sphingosine and derivates thereof have appeared in literature[15] of which the first was reported by Shapiro *et al.* in 1954.[16] In the bulk of the synthetic approaches the hydrophilic part of the sphingosine is condensed with a lipophilic tail. There are two published methods that describe the synthesis of sphingosine by modification of phytosphingosine.[17,18] One of these methods was described in **Chapter 2** of this thesis. For the synthesis of the hydrophilic part, there are many reports starting from carbohydrate sources having a D-*threo*-diol system, which is transformed into the D-*erythro* configuration present in sphingosines.[19-23] Other chirally predefined sources such as D-tartaric acid have also been used in this context.[24] Chiral auxiliaries that produce asymmetric induction have also gained interest in the synthesis of sphingosines and may best be exemplified by the use of the Evans template.[25] L-Serine is the biological precursor for the biosynthesis of D-*erythro*-sphingosine[26] and it is not surprising that many recent studies begin with L-serine, via the formation of Garners aldehyde[27], for the chemical synthesis of D-*erythro*-sphingosine.[28-31]

A retrosynthesis of a $^{13}C_5$-labeled sphingolipid **A** is presented in Scheme 1. It is based on the cross-metathesis of the D-*erythro*-configured alkene **B** and the labeled unsaturated lipid tail **C**, which can be synthesized with the requirements stated above taken into account. The presented approach was selected for a number of reasons. First, this strategy has the advantage that the stereochemistry of the hydrophilic "head" already is defined at the stage where the labeled part is introduced to the sphingolipid. Second, cross metathesis is a commonly used synthetic technique for the assembly of sphingolipid derivatives. Additionally, synthesis of a $^{13}C_5$-labeled olefin is attractive, since it can be applied for the formation of a wide range of ^{13}C-labeled long-chain fatty acids or (glyco)sphingolipids via the cross-metathesis reaction[32].

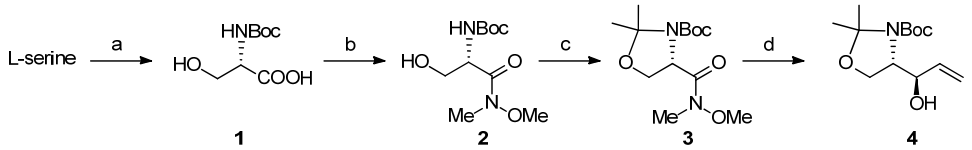
Scheme 1. Retrosynthetic analysis of a partially protected [$^{13}C_5$]-D-*erythro*-sphingosine.



Synthesis of $^{13}C_5$-D-*erythro*-sphingosine

The hydrophilic part of D-*erythro*-sphingosine was synthesized from L-serine according to literature procedures with only minor modifications (Scheme 2). The primary amine of L-serine was protected as a *tert*-butyl carbamate, followed by transformation of the carboxylic acid into Weinreb amide **2**, using EDCI as a coupling reagent. Oxazolidine **3** was formed by reaction with 2,2-dimethoxypropane, using $BF_3{\cdot}Et_2O$ as Lewis acid.[33] Additions of vinyl organometallic reagents to the Garner aldehyde[27] have been extensively investigated, presenting vinyl lithium as the reagent of choice for achieving high *anti*-selectivity.[34] Vinyl lithium is preferably synthesized from tetravinyl tin by the addition of *n*-BuLi.[35] The *in situ* formed vinyl lithium was immediately used in a reaction with crude Garner aldehyde, which had been generated from Weinreb amide **3** by $LiAlH_4$ reduction, producing the hydrophilic cross-metathesis partner **4** in a 4:1 *anti*:*syn* relationship.[36]

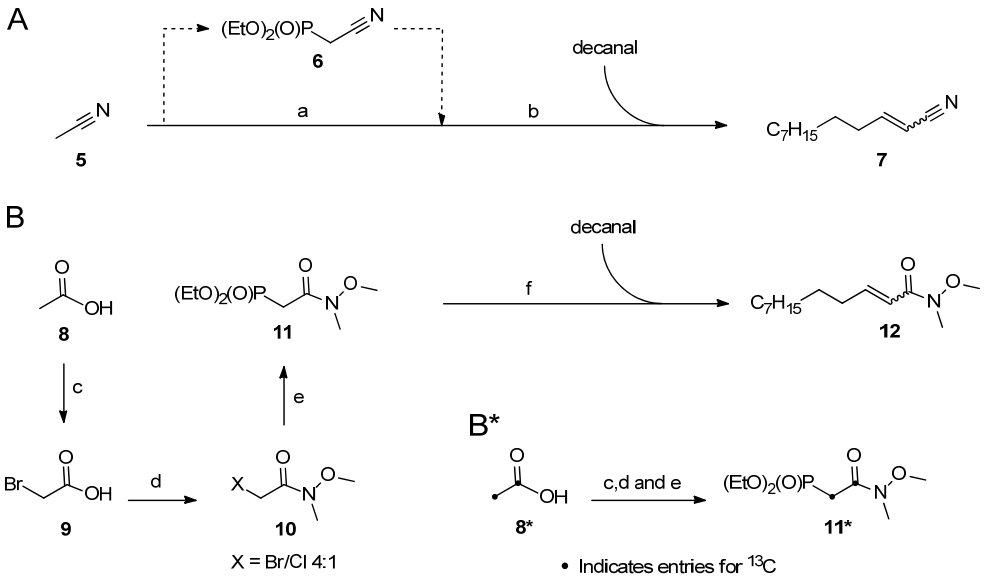
Scheme 2. Synthesis of an *L*-serine derived cross-metathesis partner.



Reagents and conditions: [a] Boc_2O, NaOH aq., 0 °C to r.t., 3 h, quant. [b] EDCI, HN(OMe)Me·HCl, NMM, −15 °C, 1 h, 94%. [c] DMP, $BF_3{\cdot}Et_2O$, acetone, r.t., 30 min., 92%. [d] i) **3**, $LiAlH_4$, THF, 0 °C, 45 min.; ii) tetravinyl tin, THF, *n*-BuLi, −78 °C then r.t., 1 h; then addition to the generated Garner aldehyde, THF, −78 °C to r.t., 20 h. 49%.

For synthesis of the fatty olefin **20*** (Scheme 4), addition of the five ^{13}C-labels in a 1+2+2 approach is attractive due to the availability of suitable and relatively inexpensive ^{13}C-labeled chemical reagents. Potassium [^{13}C]-cyanide is an affordable reagent at (10 euro/mmol)[37] and can be used to introduce a nitrile to an alkyl chain. The nitrile functionality can then be reduced/hydrolyzed to an aldehyde, which is amendable for various elongation reactions. There are two commercially available $^{13}C_2$-labeled chemicals that can be used (after modification) in two-carbon elongation reactions, namely [$^{13}C_2$]-acetonitrile (47 euro/mmol)[35] and [$^{13}C_2$]-acetic acid (21 euro/mmol)[35]. Labeled acetonitrile has previously been used in a one-pot procedure for the addition of a [$^{13}C_2$]-synthon to the growing chain of spheroidene via the Horner-Wadsworth-Emmons (HWE) reaction.[38] In this one-pot procedure, acetonitrile is first deprotonated using a strong base and then reacted with diethylphosphorochloridate, forming an intermediary C-phosphonate (**6**, Scheme 3). The C-phosphonate is then *in situ* deprotonated by a second equivalent of base, giving an ylide, which partakes in the HWE-reaction. A test sequence using the described one-pot procedure in application to a saturated fatty aldehyde was investigated (Scheme 3, Route **A**).[39] Although high conversion to the *α*,*β*-unsaturated nitrile **7** was achieved (~60%), purification of the product from the starting aldehyde proved cumbersome.

Scheme 3. Possible routes for C_2-elongation *via* the Horner-Wadsworth-Emmons reaction.



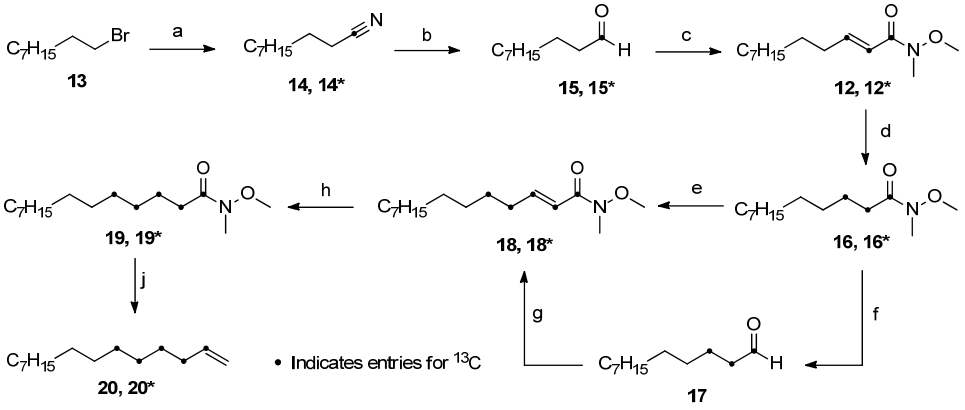
Reagents and conditions:[a] [a] *n*-BuLi, THF, −60 °C, 10 min., then diethylphosphorochloridate, −60 °C to 0 °C, 60 min. [b] LDA, 0 °C, 10 min., decanal, THF, 0 °C to r.t., 2 h, 60%. [c] TFAA, Br_2, r.t., 20 h., water, 93%, (88%)[a]. [d] i) $C_2O_2Cl_2$, DMF, DCM, 0 °C to r.t., 2 h. ii) N,O-dimethylhydroxylamine, −78 °C to r.t., 2 h., 85%, (97%)[a]. [e] triethylphosphite, 150 °C, 3 h, 94%, (95%)[a]. [f] **11**, *n*-BuLi, THF, 0 °C, 10 min., then decanal, THF, 0 °C to r.t., 20 h., 89%, 10:1 *E*/*Z*.

In an effort to overcome this problem, an alternative approach for the C_2-elongation reaction was examined (Scheme 3, Route **B**). In this route, acetic acid was first transformed into bromoacetic acid **9** *via* a modification of the Hell-Volhard-Zelinsky reaction.[40] Bromo-*N*-methoxy-*N*-methylacetamide has previously been synthesized by addition of *N*,*O*-dimethylhydroxylamine to bromoacetyl bromide.[41] Bromoacetic acid **9** was therefore activated with oxalylchloride,[42] followed by addition of *N*,*O*-dimethyl-hydroxylamine in a one-pot fashion to minimize possible hydrolysis of the intermediate acid chloride. NMR-analysis showed the formation of two products that were identified as the bromo- and chloro-*N*-methoxy-*N*-methylacetamide. The latter is presumably the result of an S_N2 displacement of the bromide by *in situ* released chloride. Since both the bromo- and chloroacetyl derivates can be used as substrates in the ensuing Michaelis-Arbuzov reaction,[43] they were not separated but isolated as a 4:1 mixture. Bromo/chloro Weinreb amide **10** was then heated with triethylphosphite forming the C_2-elongation reagent **11** in 74% yield over four steps. Utilization of the C_2-elongation reagent **11** in a HWE-reaction with decanal produced the *α*,*β*-unsaturated Weinreb amide **12** in 89% yield with a 10:1 *E*/*Z*-ratio. Satisfactorily, both the *E*- and *Z*-isomers were easily separated from the starting decanal.

[a] Yields in parentheses display the yield when labeled reagents are used.

Having established the most favourable elongation route for fatty aldehydes, the synthesis of $^{13}C_5$-olefin **20*** was undertaken. Following the same reaction sequence as for the non-labeled material, [$^{13}C_2$]-acetic acid **8*** was transformed into $^{13}C_2$-elongation reagent **11*** in 81% overall yield (Scheme 3, Route **B***). 1-Bromononane **13** was then heated with the $^{13}C_1$-labeled potassium cyanide in ethanol/water to give [1-$^{13}C_1$]-decanitrile **14*** (Scheme 4). Reduction of labeled decanitrile **14*** with DiBAL gave [1-$^{13}C_1$]-decanal **15*** suitable for elongation by the HWE reaction. Utilization of the labeled C-phosphonate **11*** in a HWE-reaction with $^{13}C_1$-aldehyde **15*** produced the *α,β*-unsaturated $^{13}C_3$-Weinreb amide **12*** in a 10:1 *E*/*Z*-ratio. Reduction of the double bond in **12*** by catalytic hydrogenation formed the saturated $^{13}C_3$-Weinreb amide **16***. In a test reaction sequence Weinreb amide **16** was reduced with either $LiAlH_4$ or DiBAL producing dodecanal **17**, with $LiAlH_4$ as the superior reducing agent (75% vs. 66%). Dodecanal **17** was then exposed to a second HWE-reaction giving Weinreb amide **18** in 71% yield over two steps. Alternatively, reduction and elongation of the non-labeled **16**, without isolation of the intermediate dodecanal **17** produced the *α,β*-unsaturated Weinreb amide **18** in an improved 83% yield. Following the optimised procedure $^{13}C_3$-labeled Weinreb amide **16*** was transformed into the *α,β*-unsaturated $^{13}C_5$-labeled Weinreb amide **18*** without isolation of the intermediate [1,2,3-$^{13}C_3$]-dodecanal.

Scheme 4. Assembly of $^{13}C_5$-labeled 1-pentadecene.



Reagents and conditions:[a] [a] KCN, EtOH/H_2O, 80 °C, 20 h, 95%, (95%)[a]. [b] DiBAL, THF, 0 °C to r.t., 2.5 h., acidic work up, 86%, (92%)[a]. [c] i) **11**, *n*-BuLi, THF, 0 °C, 10 min., ii) decanal, THF, 0 °C to r.t., 20 h., 89%, (87%)[a], 10:1 *E*/*Z*. [d] Pd-C, H_2 (g), EtOAc, r.t., 20 h., 99%, (82%)[a]. [e] i) $LiAlH_4$, THF, 0 °C, 45 min. ii) **11**, *n*-BuLi, THF, 0 °C, 10 min., iii) dodecanal, THF, 0 °C to r.t., 20 h., 83%, (77%)[a], 10:1 *E*/*Z*. [f] $LiAlH_4$, THF, 0 °C, 45 min., 75%. [g] **11**, *n*-BuLi, THF, 0 °C, 10 min., then **17**, THF, 0 °C to r.t., 20 h., 94% [h] Pd-C, H_2 (g), EtOAc, r.t., 20 h., 92%, (93%)[a]. [j] i) $LiAlH_4$, THF, 0 °C, 45 min. ii) $MePh_3PBr$, *n*-BuLi, THF, 0 °C, 10 min., iii) tetradecanal, THF, 0 °C to r.t., 20 h., 79%, (93%)[a].

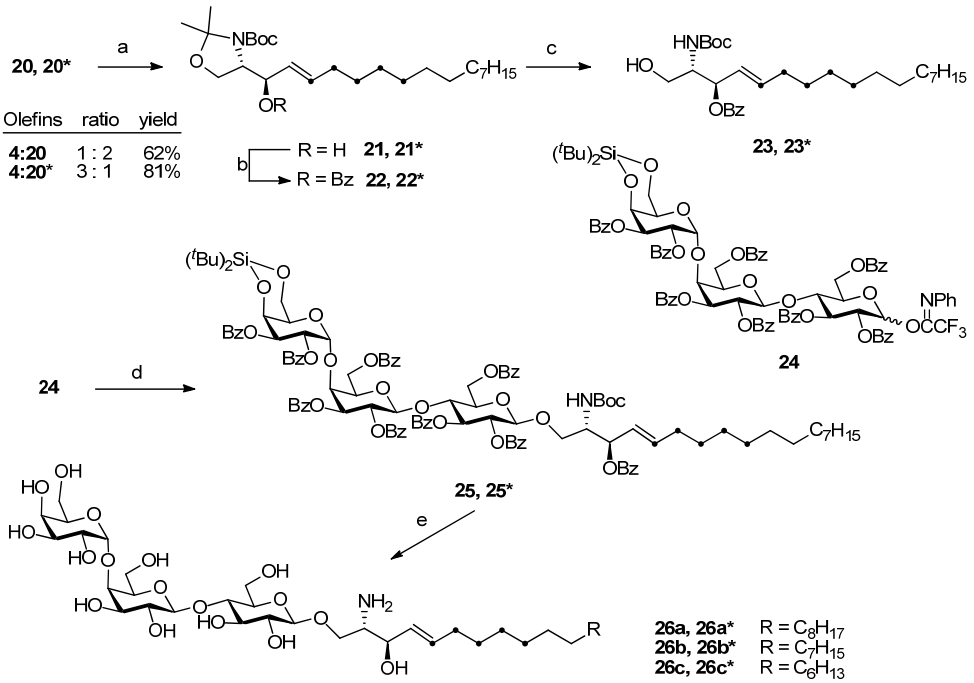
[a] Yields in parenthesis display the yield when labeled reagents are used.

The saturated $^{13}C_5$-Weinreb amide **19*** was then acquired by catalytic hydrogenation of *α,β*-unsaturated $^{13}C_5$-Weinreb amide **18*** (Scheme 4). Additional reduction of **19*** with $LiAlH_4$ produced an intermediate [1,2,3,4,5-$^{13}C_5$]-tetradecanal that immediately was used in a Wittig reaction with methyltriphenylphosphonium bromide, forming fatty $^{13}C_5$-labeled olefin **20***.

The lower cost of [$^{13}C_2$]-acetic acid as compared to [$^{13}C_2$]-acetonitrile, in combination with a high yield in the formation of HWE-reagent **11*** and easy purification of products, makes HWE-reagent **11*** a very efficient synthon for the construction of ^{13}C-labeled fatty acids.

Cross-metathesis between olefin **4** and **20** or **20*** using Grubbs 2nd generation catalyst afforded the D-*erythro*-sphingosine **21** and the $^{13}C_5$-labeled derivate **21*** in about 3.5:1 *E/Z* -ratio (Scheme 5). In light of the expensive nature of olefin **20*** a three-fold excess of olefin **4** was used in the synthesis of **21***, whereas a two-fold excess of olefin **20**[44] was used in the formation of sphingosine **21**. A slightly higher yield was achieved in the cross-metathesis reaction when olefin **4** was used in excess as compared to when olefin **20** was used in excess.

Scheme 5. Assembly of labeled and non-labeled globotriaosylsphingosine.



Reagents and conditions: [a] **4**, Grubbs 2nd generation catalyst, DCM, reflux, 6 h., 62%, (81%). [b] BzCl, DMAP, DCM/pyridine, r.t., 20 h., 84%, (92%). [c] MeOH/EtOH, pTsOH, r.t., 20 h., 54%, (63%). [d] **23** or **23***, $BF_3{\cdot}Et_2O$, DCM, 0 °C, 2 h., 60%, (55%). [e] i) HF·pyridine, THF/pyridine, r.t., 4 h., ii) NaOMe, MeOH, r.t., 20 h., iii) KOH, water, r.t., 20 h., iv) TFA (neat), 0 °C, 2 min., **26a-b-c**: 39-13-1%; **26*a-b-c**: 48-11-1%.

In **Chapter 2** a 3-*O*-benzoyl-2-azidosphingosine featured as an acceptor in the synthesis of lysoCTH. In order to minimize the number of chemical transformations with the $^{13}C_5$-labeled sphingosine it was decided to use 3-*O*-benzoyl-*N*-*tert*-butyloxycarbonyl sphingosine **23*** as acceptor in the glycosylation with *N*-phenyl trifluoroimidate **24**. $^{13}C_5$-labeled acceptor **23*** was accessed by benzoylation of **21***, followed by acidic deprotection of the isopropylidene protective group.

In a publication by Wong *et al.* globotriaosylsphingosine **25** was successfully synthesized, using a trichloroacetimidate derivate of **24** as donor and sphingosine **23** as acceptor with TMSOTf as catalyst.[45] Using the reported conditions, glycosylation of **23** with globotriaosyl *N*-phenyl trifluoroacetimidate **24** produced the lysoCTH product **25**, but with concomitant removal of the Boc-protective group. Another, commonly used Lewis acid for the activation of glycosyl trichloroacetimidates is $BF_3{\cdot}Et_2O$[46] and although this lewis acid has been shown to facilitate deprotection of *tert*-butylcarbamate protecting groups[47] it was still investigated as a activator for the *N*-phenyl trifluoroacetimidate donor **24**. The results in Table 1[a] show that near equimolar amounts of $BF_3{\cdot}Et_2O$ in comparison with the donor are required to produce the product in good yield. With $BF_3{\cdot}Et_2O$ as activator in the glycosylation reaction, no concomitant deprotection of the carbamate was observed. Furthermore, a larger excess of imidate donor compared to acceptor (Tabel 1, entry 3) produces a lower yield in the condensation reaction after purification, which arguably is a result of the difficult separation between the hydrolyzed imidate donor and lysoCTH.

Table 1. Imidate glycosylation, forming lysoCTH.

entry	donor:acceptor	$BF_3{\cdot}Et_2O$	yield
1	1.5 : 1.0	0.25	22%
2	1.5 : 1.0	1.4	49%
3	2.0 : 1.0	1.9	45%
4	1.2 : 1.0	1.1	60% (55%)

With the protected globotriaosylsphingosine derivates **25** and **25*** in hand, attention was directed to the deprotection and purification (Scheme 5). The 4,6-di-*tert*-butylsilylene was removed with hydrogen fluoride in pyridine, followed by debenzoylation with sodium methoxide in methanol. This step produces methylbenzoate that was saponified with potassium hydroxide, before removal of the *tert*-butylcarbamate by neat TFA.[48] Purification of the crude lysoCTH was then performed by RP–HPLC with mass spectrometric detection of the eluting products.

Examination of the HPLC–MS trace during purification, revealed the occurrence of not only the desired product, but also two additional peaks having shorter retention times (Figure 1). These peaks had mass differences of 14 and 28 mass units respectively, compared to the desired product (and mass differences of 15 and 30 for the labeled product). The difference in mass and retention time suggested formation of nor-homologues by elimination of one or two methylene groups.

[a] Yield in parenthesis corresponds to the yield for the formation of $^{13}C_5$-labeled lysoCTH

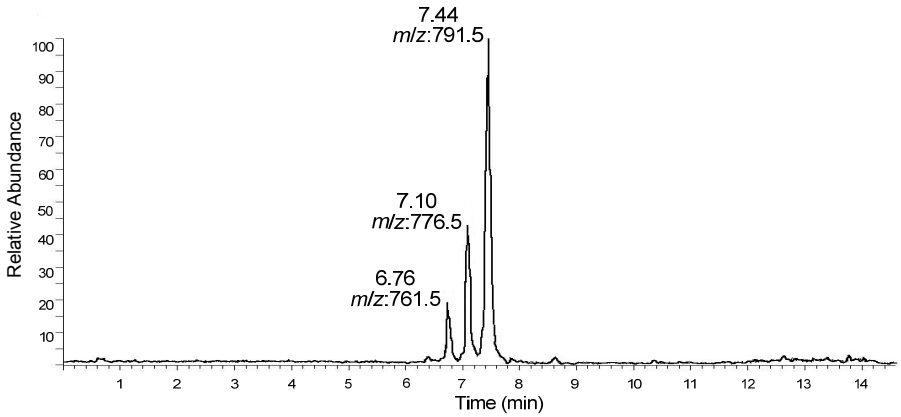


Figure 1. HPLC-MS trace of $^{13}C_5$-labeled lysoCTH **26*a** and its nor- and di-nor homologues **26*b-c**.

Analysis of the three products **26a–c** by ^{1}H and ^{13}C NMR (600 MHz) further corroborated the formation of the nor- and di-nor-homologues. Reexamination of the $^{13}C_5$-labeled and non-labeled compounds **20(*)** to **25(*)** revealed that compounds **21(*)–25(*)** consisted of the same mixture of homologues as determined for **26(*)**, while the identity and purity of $^{13}C_5$-labeled and non-labeled olefin **20(*)** were undisputed. The existence of the nor- and di-nor-homologues in compounds **21(*)–25(*)** could not be concluded by either ^{1}H- or ^{13}C-NMR alone,[49] but their existence could only be confirmed by mass spectrometry.[50] These data strongly suggest that the truncated derivates are formed during the cross-metathesis, probably by isomerization of the double bond in olefins **20** and **20*** while attached to the ruthenium catalyst.[51]

The three derivates **26a–c** produce close to identical ^{1}H-NMR and ^{13}C-NMR spectra.[52] This fact demonstrates the difficulty in determining the existence of (nor)homologues in sphingosines by NMR analysis alone. There are many articles describing the use of cross-metathesis for the synthesis of sphingosine derivates, but none of them report the formation of nor-homologues during the cross-metathesis reaction.[45,53-58] Isomerization and elimination processes during cross metathesis are well known features although the mechanisms for this side reactions have not yet been elucidated.[59,60]

When comparing the obtained product ratios of **26a–c** (39:13:1) to **26*a–c** (48:11:1), it becomes clear that the ratio between olefins **4** and **20/20***, in the cross-metathesis, had an influence on the isomerization/elimination process. It is known that terminal unhindered olefins (**20**) give rapid homodimerisation and that 2° allylic alcohols (**4**) partake much slower in a homodimerisation event, when subjected to cross metathesis conditions.[61] This is an indication of their relative ability to react with the ruthenium complex. Olefin **20*** will give a faster conversion to product, when an excess of **4** is applied in the cross metathesis. This can explain the difference in product ratios acquired in the formation of the labeled as compared to the non-labeled sphingosine structures.

The differences in 400 MHz ^{1}H-NMR between labeled **26a*** (Figure 2, **A**) and non-labeled **26a** (Figure 2, **B**) are shown in Figure 2. A ^{13}C-decoupled ^{1}H-NMR spectrum of labeled lysoCTH **26a*** (Figure 2, **C**) gives an identical spectrum to the ^{1}H-NMR spectrum of **26a** (Figure 2, **B**). This proves that compounds **26a** and the $^{13}C_5$-labeled form **26a*** have an identical chemical framework. Furthermore, the identity of globotriaosylsphingosine **26a** was confirmed by comparison of analytical data (^{1}H-NMR and HPLC–MS) from the commercially available globotriaosylsphingosine.[62]

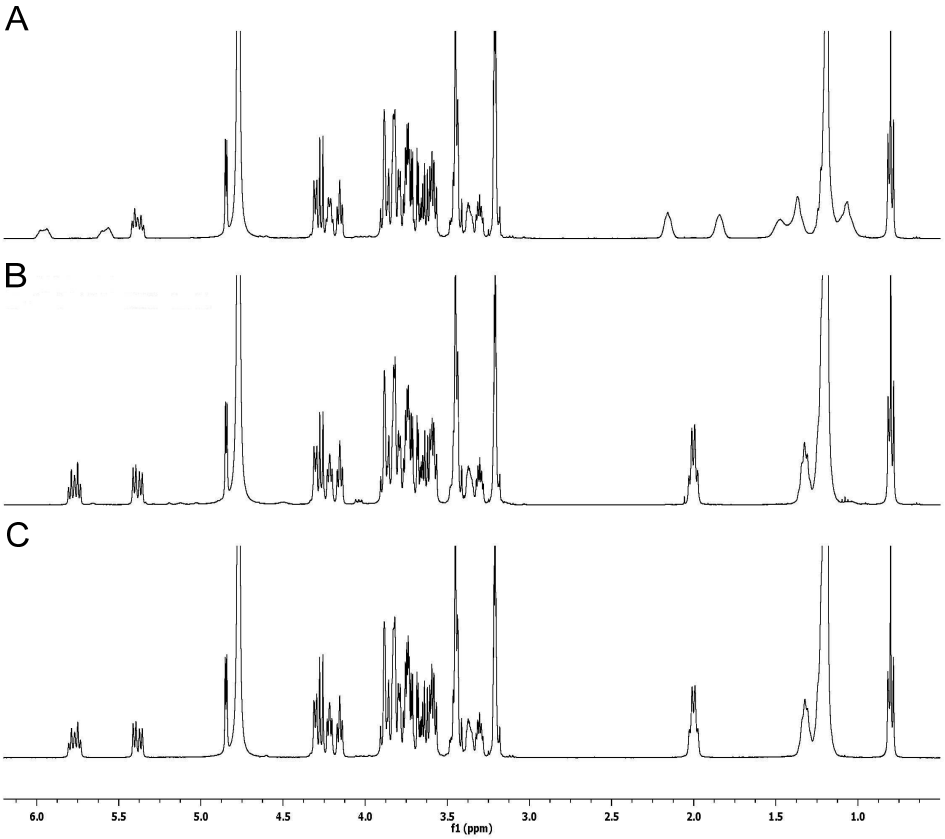


Figure 2. ^{1}H-NMR of the synthesized labeled and nonlabeled lysoCTH. **A:** 400 MHz ^{1}H-NMR (MeOD-*d*4) of labeled lysoCTH **26*a**; **B:** 400 MHz ^{1}H-NMR (MeOD-*d*4) of lysoCTH **26a**; **C:** 400 MHz ^{1}H-NMR (MeOD-*d*4) with ^{13}C-decoupling of labeled lysoCTH **26*a**.

The ^{13}C-APT-NMR of non-labeled lysoCTH **26a** is depicted in Figure 3, **A** as comparison to the ^{13}C-NMR of the labeled lysoCTH **26a*** and its nor-homologue **26b***, which are depicted in Figure 3, **B** and **C** respectively. The isomerization/elimination process during the cross metathesis is further corroborated by the difference in ^{13}C-NMR between the labeled lysoCTH **26a*** and its nor-homologue **26b*** which clearly shows the elimination of a ^{13}C-labeled methylene in the nor-homologue **26b*** (Figure 3, **C**).

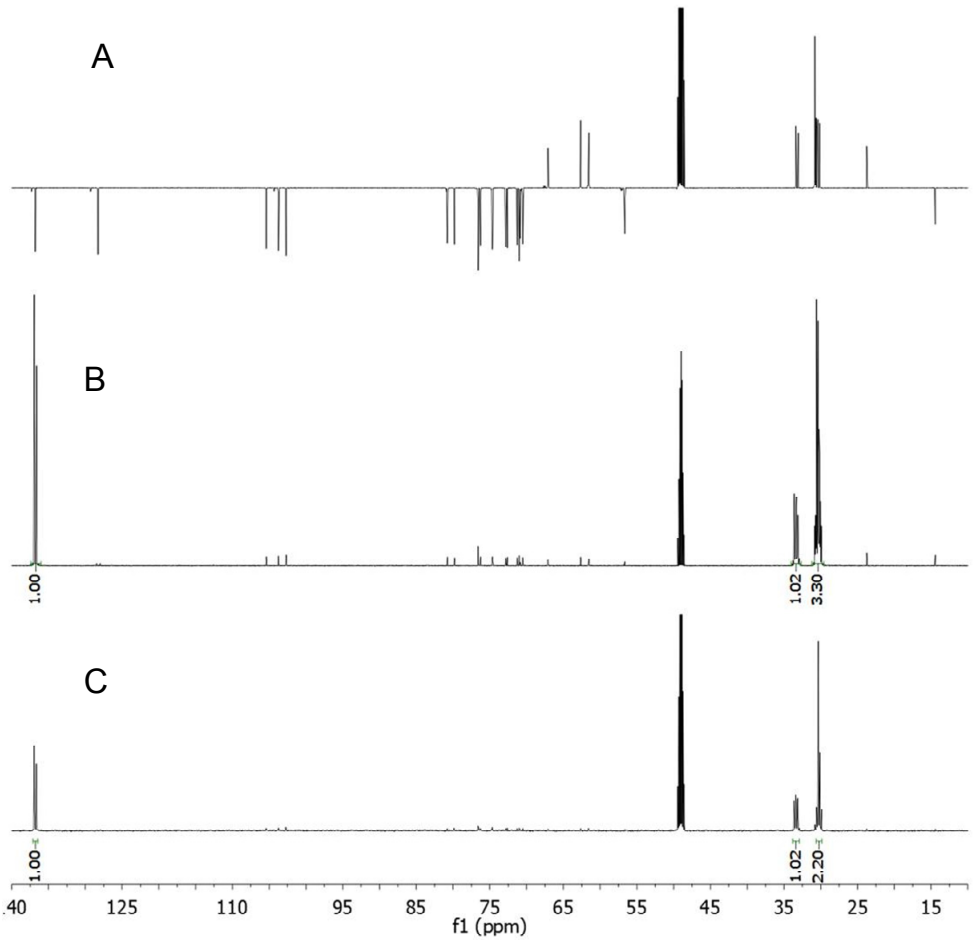


Figure 3. ^{13}C-NMR of the synthesized labeled and nonlabeled lysoCTH. **A:** ^{13}C-NMR APT (MeOD-*d*4) of the non-labeled lysoCTH **26a**; **B:** ^{13}C-NMR (MeOD-*d*4) of the labeled lysoCTH **26a***; **C:** ^{13}C-NMR (MeOD-*d*4) of the labeled lysoCTH nor-homologue **26b***.

The synthesized $^{13}C_5$-globotriaosyl-D-*erythro*-sphingosine is currently being used in clinic to quantify lysoCTH levels in the plasma of Fabry patients. The difference in lysoCTH levels between a control (Figure 4, **A**) and Fabry patient (Figure 4, **B**) are depicted in Figure 4. In these experiments, the test samples are 'spiked' with labeled **26a*** as internal standard and then applied to an HPLC–MS equipped with a quadrupole mass spectrometer. Both the endogenous lysoCTH and the synthesized substrate **26a*** experience the same retention time on column (~3.1 min.) showing their identical physiochemical properties.[63] The left graphs in Figure 4 show the peaks corresponding to the mass of the endogenous lysoCTH, while the right side indicates the peaks with the mass of the internal standard. The globotriaosylsphingosine levels between the control plasma and plasma from Fabry patient are quite striking as can be observed in Figure 4.

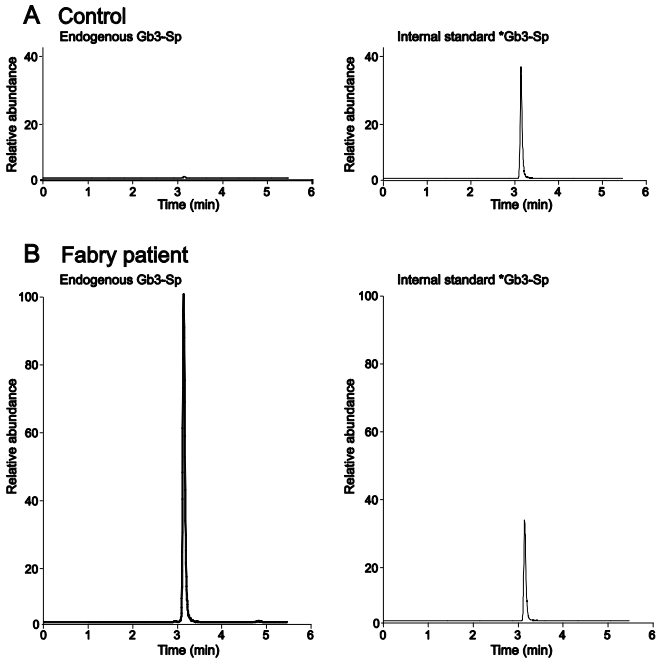


Figure 4. Monitoring of lysoCTH plasma levels with **26a*** as internal standard, by triple quadrupole mass spectrometry. **A**: Control. **B**: Fabry patient.The relative intensities of the masses corresponding to endogenous and labeled lysoCTH are shown.

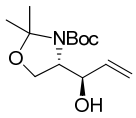
The synthesized $^{13}C_5$-labeled lysoCTH represents a well-defined internal standard, which stand in contrast to the less defined non-labeled commercially available globotriaosylsphingosine. A well-defined internal standard is important for a correct monitoring of and screening for Fabry patients. The involvement and correlation of lysoCTH levels in Fabry patients is a requisite for the ability to use this substance as a biomarker for Fabry's disease. The ability to accurately monitor its levels can therefore be of great importance for the therapeutic management of patients and their well-being.[10]

Conclusion

The synthesis of a $^{13}C_5$-labeled D-*erythro*-sphingosine was successfully executed utilizing a high-yielding procedure for the elongation of fatty aldehydes. Undesired methylene elimination(s) during a cross-metathesis reaction was discovered in the synthesis of D-*erythro*-sphingosine, which unfortunately lowers the yield and efficiency of the synthesis. The labeled sphingosine was then successfully glycosylated with globotriaosyl imidate utilizing $BF_3{\cdot}Et_2O$ as catalyst, forming a fully protected $^{13}C_5$-labeled lysoCTH. Successful deprotection followed by RP–HPLC purification produced the target $^{13}C_5$-labeled lysoCTH as well as the nor- and di-nor-homologues. The structure of the $^{13}C_5$-globotriaosylsphingosine was firmly established by NMR and MS techniques. The $^{13}C_5$-labeled lysoCTH is now successfully used as an internal standard for the monitoring of lysoCTH levels in Fabry patients.

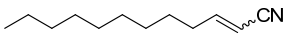
Experimental section

General Procedures and Material: Commercially available reagents and solvents (Acros, Fluka or Merck) were used as received unless stated otherwise. DCM and THF were freshly distilled, before use, over P_2O_5 and Na/benzophenone respectively. Triethylamine was destilled over calcium hydride and stored over KOH. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water was removed from starting compounds by coevaporation with DCE, dioxane and/or toluene. All moisture sensitive reactions were performed under an atmosphere of argon. Molecular sieves 3Å were flamedried prior to use. Liquid column chromatography was performed using forced flow of the indicated solvent systems on Screening Devices Silica gel 60 (40–63 µm mesh). Size exclusion chromatography was performed on Sephadex LH20 (eluent MeOH/DCM, 1:1). Analytical TLC was performed on aluminium sheets, pre-coated with silica gel (Merck, silica gel 60, F_{254}). Compounds were visualized with UV absorption (254 nm), by spraying with either 20% H_2SO_4 in ethanol, or ammonium molybdate/cerium sulphate solution [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_6\cdot 2H_2O$ (10 g/L), 10% sulphuric acid in ethanol], or phosphormolybdic acid in EtOH (150 g/L) followed by charring (~150 °C) or by spraying with an aqueous solution of potassium permanganate [$KMnO_4$ (20 g/L), K_2CO_3 (10 g/L)]. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter (Sodium D-line, λ = 589 nm). ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 (^1H) and 100.6 (^{13}C) MHz, Bruker AV 500 MHz spectrometer at 500.0 (^1H) and 125.1 (^{13}C) MHz or Bruker AV 600 MHz spectrometer at 600.0 (^1H) and 150.1 (^{13}C) MHz respectively. Chemical shifts are reported as δ values (ppm) and directly referenced to TMS (0.00 ppm) in $CDCl_3$ or *via* the solvent residual peak (D_2O). Coupling constants (*J*) are given in Hz and all ^{13}C spectra are proton decoupled. NMR assignments were made using COSY and HSQC and in some cases TOCSY experiments. LC-MS analyses were performed on a LCQ Advantage Max (Thermo Finnigan) equipped with a Gemini C_{18} column (Phenomenex, 50 x 4.6 mm, 3µ), utilizing the following buffers: A: H_2O, B: acetonitrile and C: 1.0% $TFA_{(aq)}$. HPLC-MS purifications were performed on a Agilent Technologies 1200 Series automated HPLC system with a Quadropole MS 6130, equipped with a semi-preparative Gemini C_{18} column (Phenomenex, 250 x 10.00, 5 µ). Products were eluted using the following buffers: A: 0.2% $TFA_{(aq)}$, B: acetonitrile (HPLC-grade), 5 mL/min. Purified products were lyophilised on a CHRIST ALPHA 2–4 LD_{PLUS} to remove water and traces of buffer salts.

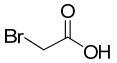


(2*S*,3*R*)-2-Amino-*N*-(*tert*-butyloxycarbonyl)-1,3-dihydroxy-1,2-*O*,*N*-isopropylidene-4-pentene (4) Weinreb amide **3**[33] (23 g, 0.10 mol, 1.0 eq) was dissolved in anhydrous THF (400 mL) and cooled to 0 °C. Lithium aluminum hydride (4 M in THF) (15 mL, 60 mmol, 0.6 eq) was added at 0 °C and the mixture was stirred at this temperature for 45 minutes. The mixture was then cooled to –20 °C and quenched with sat. aq. potassium hydrogen sulphate (300 mL). Diethylether (750 mL) was added and the reaction was stirred violently for 30 minutes. The ether layer was then dried by addition of magnesium sulphate followed by sodium sulphate. The solids were removed by filtration and washed with diethylether. The mother liqour was concentrated *in vacuo*, coevaporated once with anhydrous toluene (100 mL) and then used without further purification. Tetravinyltin (9.5 g, 7.7 mL, 42 mmol, 0.42 eq) was dissolved in anhydrous THF (250 mL) under an atmosphere of argon and cooled to −78 °C, followed by slow addition of *n*-butyllithium (1.6 M in hexanes) (0.10 L, 0.16 mol, 1.6 eq). The reaction flask was then removed from the cooling bath and stirred at room temperature for 1 hour. The crude Garners aldehyde was dissolved in anhydrous THF (100 mL) and cooled to –78 °C, followed by dropwise addition of the generated vinyl lithium. The reaction was then stirred over night, while reaching room temperature. The reaction mixture was then dissolved in EtOAc (500 mL)

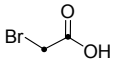
and washed with sat. aq. $NaHCO_3$ (500 mL) and brine (500 mL). The aqeous layers were then washed with EtOAc (500 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude reaction mixture was then dissolved in acetonitrile (100 mL) and washed with hexanes (100 mL x2). The hexanes was then extracted with acetonitrile (100 mL x2) and the combined acetonitrile layers were concentrated *in vacuo*, giving a yellow oil. Purification by column chromatography (8% EtOAc in petroleum ether) produced the title compound as a clear oil (12.6 g, 49 mmol, 49%) and its 2*S*,3*S* counterpart (syn adduct) in (3.2 g, 12 mmol, 12%) yield. NMR-data are in agreement with literature precedence. **2*S*,3*R*:** $[\alpha]_D^{22}$ −19 (C = 1.0, $CHCl_3$); Literature: $[\alpha]_D^{20}$ −11 (C = 0.8, $CHCl_3$)[64], $[\alpha]_D^{25}$ −19 (C = 0.8, $CHCl_3$)[65], $[\alpha]_D^{25}$ −38 (C = 1.8, $CHCl_3$)[66].



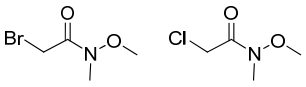
(*E*/*Z*)-Dodec-2-enenitrile (7) Acetonitrile (0.27 mL, 5.3 mmol, 1.05 eq) was dissolved in anhydrous THF (10 mL) and and cooled to −60 °C, before addition of *n*-butyllithium (1.6M in hexanes) (3.3 mL, 5.3 mmol, 1.05 eq). After stirring for 20 minutes LDA (1.6M in hexanes) (3.3 mL, 5.3 mmol, 1.05 eq) was added followed by diethylphosphorochloridate (0.76 mL, 5.3 mmol, 1.05 eq) dissolved in anhydrous THF (5 mL). The solution was then allowed to warm to 0 °C over 1 hour. Decanal (0.78 g, 5.0 mmol, 1.0 eq) was dissolved in anhydrous THF (5 mL) and was dropwise added to the phosphonate carbanion and was left stirring at roomtemperature over night. The mixture was then transferred to an extraction funnel with diethyl ether (50 mL) and washed with water (100 mL) and brine (100 mL). The water layers were extracted with ether (2 x 100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (2–20% EtOAc in petroleum ether) produced the title compounds **7E/7Z** (0.53 g, 3.0 mmol, 59 %). R_f **7E/7Z** = 0.26; R_f decanal = 0.22 (20% DCM in petroleum ether); Analytical data are in agreement with the literature presidence.[67]



2-Bromoacetic acid (9) Trifluoroacetic anhydride (12.5 mL, 90 mmol, 3.0 eq) was slowly added to acetic acid (1.8 g, 3.0 mmol, 1.0 eq), under stirring. Bromine (1.6 mL, 32 mmol, 1.05 eq) was added and the reaction was then stirred at room temperature for 20 hours. The reaction mixture was cooled to 0 °C followed by addition of water (1.9 mL, 105 mmol, 3.5 eq). Excess bromine was removed by a flow of argon. The crude mixture was then dissolved in toluene (50 mL) and concentrated *in vacuo*. The latter procedure was repeated two times giving the title product as an off-white solid without further purification (3.9 g, 28 mmol, 93 %). Analytical data are in agreement with the literature presidence.[68]

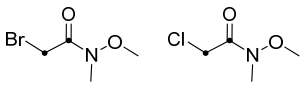


[$^{13}C_2$]-2-Bromoacetic acid (9*)[40] Trifluoroacetic acid (67 mL, 0.48 mol, 3.0 eq) was slowly added to [1,2-$^{13}C_2$]-acetic acid (10 g, 0.16 mol, 1.0 eq), under stirring. Bromine (8.3 mL, 0.16 mmol, 1.0 eq) was added and the reaction was then stirred at room temperature for 20 hours. The reaction mixture was cooled to 0 °C followed by addition of water (10 mL, 0.56 mol, 3.5 eq). Excess bromine was removed by a flow of argon. The crude mixture was then dissolved in toluene (200 mL) and concentrated *in vacuo*. This procedure was repeated two times giving the title product as an off-white solid without further purification (23 g, 0.14 mmol, 88 %). Analytical data are in agreement with the literature presidence.[40]

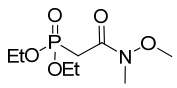


2-Bromo-*N*-methoxy-*N*-methylacetamide and 2-Chloro-*N*-methoxy-*N*-methylacetamide (10) 2-Bromoacetic acid (1.4 g, 10 mmol, 1.0 eq) was dissolved in anhydrous DCM (10 mL), put under an atmosphere of argon and cooled to 0 °C. Oxalyl chloride (1.8 mL, 20 mmol, 2.0 eq) was added followed by a drop of DMF. The reaction was then left stirring under a flow of argon at ambient temperature. When gas evolution stopped (about two hours), the reaction was concentrated *in vacuo* (10–15 °C, 180 mbar). The residue was dissolved in anhydrous DCM (40 mL) and cooled to −70 °C. *N*,*O*-Dimethylhydroxylamine (1.5 mL, 20 mmol, 2.0 eq) dissolved

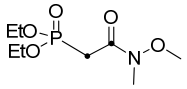
in anhydrous DCM (30 mL) was slowly added to the acylchloride at −70 °C. The reaction was left stirring, reaching room temperature over two hours and was then stirred at room temperature for an additional 30 minutes. The solids were filtered over a Whatmann paper and washed with DCM. The mother liquor was concentrated *in vacuo* and purified by column chromatography (10–40% EtOAc in petroleum ether), giving the title products in a 2.5:1 ratio (as measured by NMR) as a clear oil (1.4 g, 8.5 mmol, 85 %). R_f = 0.35 (30% EtOAc in petroleum ether); **2-Bromo-*N*-methoxy-*N*-methylacetamide:** ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.02 (s, 2 H, H-2), 3.80 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.5 (C=O), 61.6 ($CH_{3\text{-}OMe}$), 32.5 ($CH_{3\text{-}NMe}$), 25.1 (CH_2). **2-Chloro-*N*-methoxy-*N*-methylacetamide:** ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.25 (s, 2 H, H-2), 3.76 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.5 (C=O), 61.6 ($CH_{3\text{-}OMe}$), 40.7 (CH_2), 32.5 ($CH_{3\text{-}NMe}$).



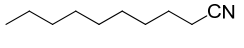
[1,2-$^{13}C_2$]-2-Bromo-*N*-methoxy-*N*-methylacetamide and [1,2-$^{13}C_2$]-2-Chloro-*N*-methoxy-*N*-methylacetamide (10*) [$^{13}C_2$]-2-Bromoacetic acid (8.5 g, 60 mmol, 1.0 eq) was dissolved in anhydrous DCM (100 mL), put under an atmosphere of argon and cooled to 0 °C. Oxalyl chloride (10.5 mL, 0.12 mol, 2.0 eq) was added followed by a drop of DMF. The reaction was then left stirring under a flow of argon at room temperature. When gas evolution stopped (about two hours), the reaction was concentrated *in vacuo* (10–15 °C, 180 mbar). The residue was dissolved in anhydrous DCM (40 mL) and cooled to −70 °C. *N*,*O*-Dimethylhydroxylamine (12 mL, 0.17 mol, 2.8 eq), dissolved in anhydrous DCM (30 mL) was slowly added to the acylchloride at −70 °C and then left stirring, reaching room temperature over two hours. The reaction was then stirred at room temperature for 30 minutes. The solids were filtered over a Whatmann paper and washed with DCM. The mother liquor was concentrated *in vacuo* and purified by column chromatography (10–40% EtOAc in petroleum ether), giving the title products in a 4:1 ratio (as measured by NMR) as a clear oil (10.3 g, 58 mmol, 97 %). R_f = 0.35 (30% EtOAc in petroleum ether); **[1,2-$^{13}C_2$]-2-Bromo-*N*-methoxy-*N*-methylacetamide:** ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.01 (dd, 2 H, *J* = 154.0, 3.6 Hz, H-2), 3.80 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.5 (d, *J* = 58.5 Hz, C=O), 61.6 ($CH_{3\text{-}OMe}$), 32.5 ($CH_{3\text{-}NMe}$), 25.1 (d, *J* = 58.4 Hz, CH_2); HRMS Calcd for [$C_2{}^{13}C_2H_8NO_2Br$ + H]$^+$: 183.9878, found 183.9877. **[1,2-$^{13}C_2$]-2-Chloro-*N*-methoxy-*N*-methylacetamide:** ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.25 (dd, 2 H, *J* = 152.3, 4.4 Hz, H-2), 3.76 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.5 (d, *J* = 57.2, C=O), 61.6 ($CH_{3\text{-}OMe}$), 40.7 (d, *J* = 57.7 Hz, CH_2), 32.5 ($CH_{3\text{-}NMe}$); HRMS Calcd for [$C_2{}^{13}C_2H_8NO_2Cl$ + H]$^+$: 140.0383 found 140.0381.



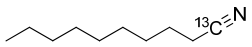
Diethyl-(*N*-methoxy-*N*-methylcarbamoylmethyl)phosphonate (11) 2-Bromo/2-Chloro-*N*-methoxy-*N*-methylacetamide (1.4 g, ~8.4 mmol, 1.0 eq) and triethylphosphite (1.5 mL, 8.6 mmol, 1.05 eq) were put in a round bottom flask equipped with an extended neck and heated for three hours at 150 °C. The crude mixture was cooled down and directly purified by column chromatography (30–50 % acetone in petroleum ether), giving the title product as a clear oil (1.9 g, 7.9 mmol, 94 %). R_f = 0.20 (40% acetone in petroleum ether, spraying with ammonium molybdate/cerium sulphate solution and charring gives a weak white spot that gives an intense blue colour after one hour). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.24 – 4.14 (m, 4 H, $CH_{2\text{-}OEt}$ x2), 3.79 (s, 3 H, $CH_{3\text{-}OMe}$), 3.22 (s, 3 H, $CH_{3\text{-}NMe}$), 3.17 (d, 2 H, *J* = 21.9, H-2), 1.35 (tm, 6 H, *J* = 7.1 Hz, $CH_{3\text{-}OEt}$ x2); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.0 (C=O), 62.5, 62.4 ($CH_{2\text{-}OEt}$ x2), 61.4 ($CH_{3\text{-}OMe}$), 30.7 ($CH_{3\text{-}NMe}$), 29.2 (C-2), 16.28, 16.26 ($CH_{3\text{-}OEt}$ x2); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 21.1; IR (neat): 2984, 1656, 1424, 1381, 1251, 1018, 961, 790, 468 cm^{-1}; HRMS Calcd for [$C_6{}^{13}C_2H_{18}NO_5P$ + Na]$^+$: 262.0815, found 262.0816.



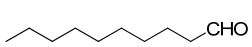
Diethyl-([1,2-$^{13}C_2$]-*N*-methoxy-*N*-methylcarbamoylmethyl) phosphonate (11*) [1,2-$^{13}C_2$]-2-Bromo/Chloro-*N*-methoxy-*N*-methylacetamide (10.3 g, 58 mmol, 1.0 eq) and triethylphosphite (10.5 mL, 60 mmol, 1.05 eq) was put in a round bottom flask equipped with an 15 cm air-cooled condenser and heated for three hours at 150 °C. The crude mixture was cooled down and directly purified by column chromatography (30–50 % acetone in petroleum ether), giving the title product as a clear oil (13.7 g, 57 mmol, 95 %). R_f = 0.20 (40% acetone in petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.24 – 4.13 (m, 4 H, $CH_{2\text{-OEt}}$ x2), 3.79 (s, 3 H, $CH_{3\text{-OMe}}$), 3.22 (s, 3 H, $CH_{3\text{-NMe}}$), 3.16 (ddd, 2 H, *J* = 129.8, 21.9, 6.6 Hz, H-2), 1.35 (t, 6 H, *J* = 7.1 Hz, $CH_{3\text{-OEt}}$ x2); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.5 (dd, *J* = 53.1, 4.5 Hz, C=O), 62.0, 61.9 ($CH_{2\text{-OEt}}$ x2), 60.9 ($CH_{3\text{-OMe}}$), 31.57 ($CH_{3\text{-NMe}}$), 30.9 (dd, *J* = 136.1, 53.1 Hz, H-2), 15.82, 15.76 ($CH_{3\text{-OEt}}$ x2); IR (neat): 2984, 1658, 1423, 1381, 1253, 1018, 961, 789 cm^{-1}; HRMS Calcd for $[C_6{}^{13}C_2H_{18}NO_5P + H]^+$: 242.1063, found 242.1064.



Decanitrile (13) Potassium cyanide (2.05 g, 31.5 mmol, 1.05 eq) was added to a solution of 1-bromo-nonane (6.21 g, 30 mmol, 1.0 eq) in a mixture of ethanol/water (9:1, 60 mL) and then heated at 80 °C over night. The reaction was then diluted with ether (200 mL) and washed with water (2 x 200 mL) and brine (150 mL). The waterlayers were extracted with Ether (200 mL) and the combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (0–3% EtOAc in petroleum ether) gave the title compound as a clear oil (4.37 g, 28.5 mmol, 95 %). R_f = 0.23 (3% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.33 (t, 2 H, *J* = 7.1 Hz, H-2), 1.66 (m, 2 H, H-3), 1.45, (m, 2 H, H-4), 1.34 – 1.22 (m, 10 H, H-5 to H-9), 0.88 (t, 3 H, *J* = 6.6 Hz, H-10); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 119.8 (C≡N), 31.7, 29.2, 29.1, 28.7 (CH_2 x4), 28.6 (C-4), 25.3 (C-3), 22.6 (CH_2), 17.1 (C-2), 14.0 (C-10); IR (neat): 2926, 2856, 1467, 1427, 723 cm^{-1}.

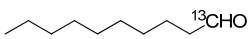


[1-$^{13}C_1$]-Decanitrile (13*) [$^{13}C_1$]-Potassium cyanide (5.00 g, 76.0 mmol, 1.0 eq) was added to a solution of 1-bromo-nonane (16.5 g, 79.0 mmol, 1.05 eq) in a mixture of Ethanol/water (9:1, 140 mL) and then heated at 80 °C over night. The reaction was cooled to room temperature and diluted with ether (500 mL) and washed with water (2 x 500 mL) and brine (400 mL). The waterlayers were extracted with ether (400 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (0–2% EtOAc in petroleum ether) gave the title compound as a clear oil (11.1 g, 72.0 mmol, 95 %). R_f = 0.23 (3% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.33 (dt, 2 H, *J* = 9.6, 7.1 Hz, H-2), 1.65 (m, 2 H, H-3), 1.44, (m, 2 H, H-4), 1.35 – 1.22 (m, 10 H, H-5 to H-9), 0.88 (t, 3H, *J* = 6.9 Hz, H-10); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 119.8 (C≡N), 31.7, 29.2, 29.1, 28.7 (CH_2 x4), 28.5 (d, *J* = 3.3 Hz, C-4), 25.3 (d, *J* = 0.4 Hz, C-3), 22.5 (CH_2), 17.0 (d, *J* = 55.8 Hz, C-2), 14.0 (C-10); IR (neat): 2925, 2856, 2194, 1467, 1425, 1378, 721 cm^{-1}; HRMS Calcd for $[C_{10}H_{19}N + H]^+$: 155.2623, found 155.1624.

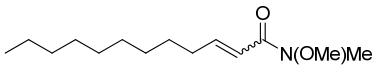


Decanal (14) Decanitrile (4.4 g, 29 mmol, 1.0 eq) was dissolved in anhydrous THF (120 mL) and cooled to 0 °C before addition of DIBAL-H (1.5 M in hexanes) (23 mL, 34 mmol, 1.2 eq). The reaction mixture was stirred at ambient temperature for 2.5 hours. The mixture was then transferred to an extraction funnel, diluted with ether (100 mL) and washed with 1 M HCl (2 x 200 mL), sat. aq. $NaHCO_3$ (200 mL). The water layers were extracted with ether (2 x 200 mL) and the combined organics were dried ($MgSO_4$), filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–10% DCM in petroleum ether) produced the title compound as a clear oil (3.9 g, 25 mmol, 86 %). R_f = 0.21 (3% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.76 (t, 1 H, *J* = 1.9 Hz, H-1), 2.42 (td, 2 H, *J* = 7.4, 1.9 Hz, H-2), 1.63 (m, 2 H, H-3), 1.36 – 1.23 (m, 12 H, H-4 to H-9), 0.88 (t, 3 H, *J* = 6.9 Hz, H-10); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 202.7 (C=O), 43.8 (C-2), 31.8,

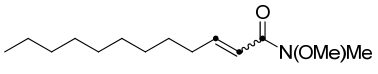
29.30, 29.28, 29.2 (CH_2 x4), 29.1 (C-4), 22.6 (CH_2), 22.0 (C-3), 14.0 (C-10); IR (neat): 2924, 2855, 1727, 1467, 723 cm^{-1}.



[1-$^{13}C_1$]-Decanal (14*) [1-$^{13}C_1$]-Decanitrile (11 g, 72 mmol, 1.0 eq) was dissolved in anhydrous THF (250 mL) and cooled to 0 °C before addition of DIBAL-H (1.5 M in hexanes) (53 mL, 79 mmol, 1.1 eq). The reaction mixture was stirred at ambient temperature for 2.5 hours. The mixture was then transferred to an extraction funnel, diluted with ether (200 mL) and washed with 1 M HCl (2 x 400 mL), sat. aq. $NaHCO_3$ (400 mL). The water layers were extracted with ether (2 x 400 mL) and the combined organics were dried ($MgSO_4$), filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–10% DCM in petroleum ether) produced the title compound as a clear oil (10.4 g, 66.1 mmol, 92 %). R_f = 0.22 (20% DCM in petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) δ: 9.76 (dt, 1 H, *J* = 169.8, 1.9 Hz), 2.42 (dtd, 2 H, *J* = 7.4, 6.2, 1.8 Hz), 1.62 (m, 2 H), 1.36 – 1.23 (m, 12 H), 0.88 (t, 3H, *J* = 6.9 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 203.0 (C=O), 43.9 (d, *J* = 38.8, C-2), 31.8, 29.35, 29.32, 29.2 (CH_2 x4), 29.1 (d, *J* = 3.4 Hz, C-4), 22.6 (CH_2), 22.0 (d, *J* = 1.6 Hz, C-3), 14.0 (C-10); IR (neat): 2922, 2855, 1728, 1466, 719 cm^{-1}.

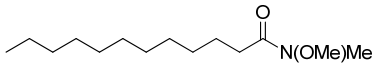


(*E*)-*N*-Methoxy-*N*-methyl-dodec-2-enamide (15E) and (*Z*)-*N*-Methoxy-*N*-methyl-dodec-2-enamide (15Z) Diethyl (*N*-Methoxy-*N*-methyl-carbamoyl-methyl)phosphonate **(11)** (2.5 g, 10.5 mmol, 1.1 eq) was dissolved in dry THF (50 mL) and cooled to 0 °C before addition of *n*-butyllithium (1.6 M in hexanes) (6.6 mL, 10.5 mmol, 1.1 eq). The reaction mixture was stirred for 10 minutes at 0 °C. Decanal (1.5 g, 9.5 mmol, 1.0 eq) dissolved in anhydrous THF (20 mL) was added to the phosphonat carbanion and the reaction was stirred at room temperature over night. The mixture was then transferred to an extraction funnel with diethyl ether (50 mL) and washed with water (100 mL) and brine (100 mL). The water layers were extracted with ether (2 x 100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (2–20% EtOAc in petroleum ether) produced the title compounds **15E** (1.85 g, 7.7 mmol, 81 %) and **15Z** (0.18 g, 0.75 mmol, 8 %) in a combined yield of 89 % as clear oils. R_f **15E** = 0.42; **15Z** = 0.64 (20% EtOAc in petroleum ether); (***E*-isomer**, **15E**) ^1H NMR (400 MHz, $CDCl_3$) δ: 6.98 (dt, 1 H, *J* = 15.4, 7.0 Hz, H-3), 6.39 (d, 1 H, *J* = 15.4 Hz, H-2), 3.70 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$), 2.23 (dt, 2 H, *J* = 7.0, 6.1 Hz, H-4), 1.51 – 1.42 (m, 2 H, H-5), 1.35 –1.23 (m, 12 H, H-6 to H-11), 0.88 (t, 3 H, *J* = 6.5 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.0 (C=O), 148.0 (C-3), 118.5 (C-2), 61.6 ($CH_{3\text{-}OMe}$), 32.5 (C-4), 32.3 ($CH_{3\text{-}NMe}$), 31.8, 29.5, 29.4, 29.2, 29.1 (CH_2 x5), 28.3 (C-5), 22.6 (CH_2), 14.0 (C-12); IR (neat): 2925, 2855, 1664, 1636, 1464, 1378, 1178, 996 cm^{-1}; HRMS Calcd for $[C_{14}H_{27}NO_2 + H]^+$: 242.2115, found 242.2116; (***Z*-isomer**, **15Z**) ^1H NMR (400 MHz, $CDCl_3$) δ: 6.23 (d, 1 H, *J* = 11.5 Hz, H-2), 6.12 (dt, 1 H, *J* = 11.5, 7.3 Hz, H-3), 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.21 (s, 3 H, $CH_{3\text{-}NMe}$), 2.62 (dt, 2 H, *J* = 7.3, 7.0 Hz, H-4), 1.47 – 1.39 (m, 2 H, H-5), 1.35 –1.22 (m, 12 H, H-6 to H-11), 0.88 (t, 3 H, *J* = 6.8 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.6 (C=O), 147.8 (C-3), 117. 9 (C-2), 61.4 ($CH_{3\text{-}OMe}$), 31.9, 31.6 ($CH_{3\text{-}NMe}$)‡, 29.53, 29.45, 29.34, 29.29, 29.27, 29.1, 22.6 (CH_2 x8), 14.1 (C-12); IR (neat): 2924, 2855, 1660, 1459, 1424, 1340, 1177, 999, 794 cm^{-1}; HRMS Calcd for $[C_{14}H_{27}NO_2 + H]^+$: 242.2115, found 242.2115.

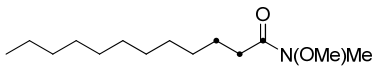


[1,2,3-$^{13}C_3$]-(*E*)-*N*-Methoxy-*N*-methyl-dodec-2-enamide (15*E) and [1,2,3-$^{13}C_3$]-(*Z*)-*N*-Methoxy-*N*-methyl-dodec-2-enamide (15*Z) Diethyl-([1,2-$^{13}C_2$]-*N*-methoxy-*N*-methylcarbamoylmethyl)phosphonate **(11*)** (10.4 g, 43 mmol, 1.1 eq) was dissolved in dry THF (200 mL) and cooled to 0 °C before addition of *n*-butyllithium 1.6 M in hexanes (26.5 mL, 42 mmol, 1.08 eq). The reaction mixture was stirred for 10 minutes at 0 °C. [1-$^{13}C_1$]-Decanal (6.2 g, 39 mmol, 1.0 eq) dissolved in anhydrous THF (40 mL) was added to the phosphonate carbanion and the reaction was stirred at room temperature over night. The

mixture was then transferred to an extraction funnel with diethyl ether (50 mL), washed with water (250 mL) and brine (200 mL). The water layers were extracted with ether (2 x 250 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (0–15% EtOAc in petroleum ether) produced the title compounds **15*E** (7.5 g, 31 mmol, 79 %) and **15*Z** (0.75 mg, 3.1 mmol, 8 %) in a combined yield of 87 % as clear oils. R_f **15*E** = 0.42; **15*Z** = 0.64 (20% EtOAc in petroleum ether); (***E*-isomer**, **15*E**) ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.98 (dm, 1 H, *J* = 153.8 Hz, H-3), 6.38 (ddd, 1 H, *J* = 160.8, 15.4, 4.1 Hz, H-2), 3.70 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$), 2.23 (m, 2 H, H-4), 1.46 (m, 2 H, H-5), 1.35 –1.23 (m, 12 H, H-6 to H-11), 0.88 (t, 3 H, *J* = 6.8 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.1 (d, *J* = 67.1 Hz, C=O), 148.0 (d, *J* = 71.6 Hz, C-3), 118.5 (dd, *J* = 71.6, 67.1 Hz, C-2), 61.6 ($CH_{3\text{-}OMe}$), 32.5 (m, C-4), 32.3 (m, $CH_{3\text{-}NMe}$), 31.9, 29.5, 29.4, 29.3 (CH_2 x4), 29.2 (d, *J* = 3.6 Hz, C-6), 28.3 (m, C-5), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2926, 5856, 1622, 1584, 1462, 1368, 1175, 993 cm^{-1}; HRMS Calcd for $[C_{11}{}^{13}C_3H_{27}NO_2H]^+$: 245.2215, found 245.2216; (***Z*-isomer**, **15*Z**) ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.22 (dd, 1 H, *J* = 161.8, 11.5 Hz, H-2), 6.11 (dm, 1 H, *J* = 152.0 Hz, H-3), 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.21 (s, 3 H, $CH_{3\text{-}NMe}$), 2.61 (m, 2 H, H-4), 1.43 (m, 2 H, H-5), 1.35 – 1.22 (m, 12 H, H-6 to H-11), 0.88 (t, 3H, *J* = 6.9 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.6 (d, *J* = 63.6 Hz, C=O), 147.8 (d, *J* = 67.1 Hz, C-3), 117. 9 (dd, *J* = 67.1, 63.6 Hz, C-2), 61.5 ($CH_{3\text{-}OMe}$), 31.9, 31.6 ($CH_{3\text{-}NMe}$)‡, 29.6, 29.5 (CH_2 x3), 29.38 (d, *J* = 4.0 Hz, C-6), 29.35 – 29.29 (m, CH_2 x2), 29.1 (m, C-3), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2925, 2855, 1618, 1459, 1334, 1178, 996, 776 cm^{-1}; HRMS Calcd for $[C_{11}{}^{13}C_3H_{27}NO_2 + H]^+$: 245.2215, found 245.2216.

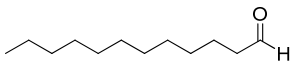


***N*-Methoxy-*N*-methyl-dodecanamide (16)** (*E*/*Z*)-*N*-Methoxy-*N*-methyl-dodec-2-enamide (**15E** and **15Z**) (1.8 g, 7.4 mmol, 1.0 eq) was dissolved in EtOAc (75 mL). The solution was purged with argon under stirring and palladium 10% on charcoal (0.39 g, 0.37 mmol, 0.05 eq), was added. The reaction mixture was then stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere over night. The palladium was removed by filtration over a Whatmann paper and rinsed with EtOAc (50 mL) followed by removal of the solvents under reduced preassure. Purification by column chromatography (5–20% EtOAc in petroleum ether) afforded the title compound as a clear oil (1.8 g, 7.4 mmol, 100%). R_f = 0.38 (20% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.18 (s, 3 H, $CH_{3\text{-}NMe}$), 2.41 (t, 2 H, *J* = 7.5 Hz, H-2), 1.62 (m, 2 H, H-3), 1.35 – 1.23 (m, 16 H, H-4 to H-11), 0.88 (t, 3 H, *J* = 6.8 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 174.4 (C=O), 61.1 ($CH_{3\text{-}OMe}$), 31.9 ($CH_{3\text{-}NMe}$), 31.8 (C-2), 29.5 (x3), 29.41, 29.36, 29.34, 29.2 (CH_2 x7), 24.6 (C-3), 22.6 (CH_2), 14.1 (C-12); IR (neat): 2923, 2854, 1668, 1464, 1383, 1176, 998, 720 cm^{-1}; HRMS Calcd for $[C_{14}H_{29}NO_2 + H]^+$: 244.2271, found 244.2272.

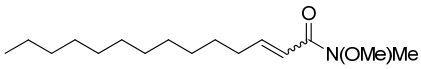


[1,2,3-$^{13}C_3$]-(E)-*N*-Methoxy-*N*-methyl-dodecanamide (16*) [1,2,3-$^{13}C_3$]-(*E*/*Z*)-*N*-Methoxy-*N*-methyl-dodec-2-enamide (**15*E** and **15*Z**) (8.3 g, 34 mmol, 1.0 eq) was dissolved in EtOAc (200 mL). The solution was purged with argon under stirring and palladium 10% on charcoal (0.72 g, 0.67 mmol, 0.02 eq), was added. The reaction mixture was then stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere over night. The palladium was removed by filtration over a Whatmann paper and rinsed with EtOAc (100 mL) followed by removal of the solvents *in vacuo*. Purification by column chromatography (5–20% EtOAc in petroleum ether) afforded the title compound as a clear oil (6.85 g, 27.8 mmol, 82%). R_f = 0.38 (20% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.18 (s, 3 H, $CH_{3\text{-}NMe}$), 2.41 (dm, 2 H, *J* = 127.3 Hz, H-2), 1.62 (dm, 2 H, *J* = 127.9 Hz, H-3), 1.35 – 1.23 (m, 16 H, H-4 to H-11), 0.88 (t, 3 H, *J* = 6.8 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 174.6 (bd, *J* = 51.5 Hz, C=O), 61.1 ($CH_{3\text{-}OMe}$), 31.9 ($CH_{3\text{-}NMe}$), 31.8 (dd, *J* = 51.5, 37.5 Hz, C-2), 29.7 – 29.1 (m, CH_2 x7), 24.6 (dd, *J* = 34.9, 1.3 Hz, C-3) , 22.6 (CH_2), 14.1

(C-12); IR (neat): 2923, 2854, 1627, 1464, 1369, 1174, 1119, 998, 722, 436 cm^{-1}; HRMS Calcd for $[C_{11}{}^{13}C_3H_{29}NO_2 + H]^+$: 247.2372, found 247.2373.

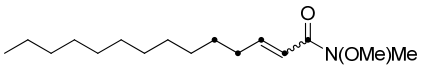


Dodecanal (17) Method A: *N*-Methoxy-*N*-methyl-dodecanamide (**16**) (1.8 g, 7.4 mmol, 1.0 eq) was dissolved in dry hexanes (40 mL) and cooled to −60 °C, before addition of DIBAL-H (1.5 M in hexanes) (5.4 mL, 8.1 mmol, 1.1 eq). The reaction was stirred for 30 minutes before being quenched with 1 M HCl (20 mL) the mixture was then transferred to an extraction funnel with EtOAc (60 mL) and washed with 1 M HCl (50 mL), sat. aq. $NaHCO_3$ (60 mL) and brine (50 mL). The water layers were extracted with EtOAc (2x 60 mL). The combined organics were dried ($MgSO_4$), filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–30% DCM in petroleum ether) produced the title compound as a clear oil (0.90 g, 4.9 mmol, 66 %). **Method B:** *N*-Methoxy-*N*-methyl-dodecanamide (**16**) (1.8 g, 7.4 mmol, 1.0 eq) was dissolved in anhydrous THF (74 mL) and cooled to 0 °C, before addition of lithium aluminium hydride (4.0 M in THF) (1.1 mL, 4.4 mmol, 0.6 eq). The reaction was stirred for 45 minutes and was then cooled to −15 °C, before addition of sat. aq. $KHSO_4$ (35 mL) and diethyl ether (100 mL). The two phase system was stirred vigorously for 30 minutes and was then dried by addition of $MgSO_4$, followed by Na_2SO_4. The solids were filtered, washed with diethylether (100 mL) and the combined eluate was then concentrated *in vacuo*. Purification by column chromatography (0–30% DCM in petroleum ether) produced the title compound as a clear oil (1.0 g, 5.5 mmol, 75 %). R_f = 0.57 (5% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.76 (t, 1 H, *J* = 1.9 Hz, H-1), 2.42 (td, 2 H, *J* = 7.4, 1.9 Hz, H-2), 1.63 (m, 2 H, H-3), 1.34 – 1.23 (m, 18 H, H-4 to H-11), 0.88 (t, 3 H, *J* = 6.8 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 202.9 (C=O), 43.9 (C-2), 31.9, 29.6, 29.6, 29.4, 29.34, 29.31, 29.2, 22.7 (C-4 to C-11), 22.1 (C-3), 14.1 (C-12); IR (neat): 2924, 2855, 1728, 1467, 1410, 1381, 722 cm^{-1}.



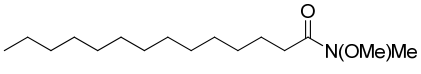
(*E*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (18E) and (*Z*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (18Z) Method A: Diethyl (*N*-Methoxy-*N*-methyl-carbamoylmethyl) phosphonate **(11)** (1.2 g, 4.9 mmol, 1.1 eq) was dissolved in anhydrous THF (25 mL) and cooled to 0 °C before addition of *n*-butyllithium (1.6 M in hexanes) (3.1 mL, 4.9 mmol, 1.1 eq). The reaction mixture was stirred for 10 minutes before addition of dodecanal (0.82 g, 4.5 mmol, 1.0 eq) dissolved in anhydrous THF (10 mL). The reaction was then stirred going to room temperature and left stirring over night. The mixture was then transferred to an extraction funnel, diluted with ether (50 mL) and washed with water (100 mL) and brine (100 mL). The water layers were extracted with ether (2 x 100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–20% EtOAc in petroleum ether) produced the title compounds **18E** (1.0 g, 3.7 mmol, 83 %) and **18Z** (0.14 g, 0.51 mmol, 11 %) in a combined yield of 94 % as clear oils. **Method B:** *N*-Methoxy-*N*-methyl-dodecanamide **(16)** (1.8 g, 7.4 mmol, 1.0 eq) was dissolved in anhydrous THF (74 mL) and cooled to 0 °C, before addition of lithium aluminium hydride (4.0 M in THF) (1.1 mL, 4.4 mmol, 0.6 eq). The reaction was stirred for 45 minutes and was then cooled to −15 °C, before addition of sat. aq. $KHSO_4$ (35 mL) and diethyl ether (100 mL). The two phase system was stirred vigorously for 30 minutes and was then dried by addition of $MgSO_4$, followed by Na_2SO_4. The solids were filtered, washed with diethylether (100 mL). The eluate was concentrated *in vacuo* giving crude dodecanal **(17)** as a clear oil which was used without further purification. Diethyl (*N*-Methoxy-*N*-methyl-carbamoylmethyl)-phosphonate **(11)** (2.0 g, 8.2 mmol, 1.1 eq) was dissolved in anhydrous THF (40 mL) and cooled to 0 °C before addition of *n*-butyllithium (1.6 M in hexanes) (5.1 mL, 8.2 mmol, 1.1 eq). The reaction mixture was stirred for 10 minutes at 0 °C. The crude dodecanal **(17)** was dissolved in anhydrous THF (10 mL) and added to the Horner-Wadsworth-Emmons reagent at 0 °C. The reaction was then stirred going to room temperature and left stirring over night. The mixture

was transferred to an extraction funnel with ether (50 mL) and washed with water (100 mL) and brine (100 mL). The water layers were extracted with ether (2 x 100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–15% EtOAc in petroleum ether) produced the title compounds **18E** (1.49 g, 5.5 mmol, 75 %) and **18Z** (0.17 g, 0.61 mmol, 8 %) in a combined yield of 83 % as clear oils. R_f **18E** = 0.39; **18Z** = 0.58 (15% EtOAc in petroleum ether). (***E*-isomer**, **18E**) ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.98 (dt, 1 H, *J* = 15.4, 7.0 Hz, H-3), 6.39 (dt, 1 H, *J* = 15.4, 1.7 Hz, H-2), 3.70 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$), 2.23 (dtd, 2 H, *J* = 7.0, 6.1, 1.7 Hz, H-4), 1.52 – 1.42 (m, 2 H, H-5), 1.36 –1.22 (m, 16 H, H-6 to H-13), 0.88 (t, 3 H, *J* = 6.5 Hz, H-14); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.1 (C=O), 148.0 (C-3), 118.6 (C-2), 61.6 ($CH_{3\text{-}OMe}$), 32.5 (C-4), 32.3 ($CH_{3\text{-}NMe}$), 31.9, 29.61, 29.59, 29.5, 29.4, 29.3, 29.2 (CH_2 x7), 28.3 (C-5), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2924, 2854, 1618, 1583, 1464, 1368, 1174, 1119, 991 cm^{-1}; HRMS Calcd for $[C_{16}H_{31}NO_2 + H]^+$: 270.2428, found 270.2429; (***Z*-isomer**, **18Z**) ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.23 (bd, 1 H, *J* = 11.5 Hz, H-2), 6.12 (dt, 1 H, *J* = 11.7, 7.3, Hz, H-3), 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.21 (s, 3 H, $CH_{3\text{-}NMe}$), 2.62 (dt, 2 H, *J* = 7.1, 7.0 Hz, H-4), 1.47 – 1.39 (m, 2 H, H-5), 1.35 –1.22 (m, 16 H, H-6 to H-13), 0.88 (t, 3 H, *J* = 6.9 Hz, H-14); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.6 (C=O), 147.8 (C-3), 117. 9 (C-2), 61.4 ($CH_{3\text{-}OMe}$), 32.0 ($CH_{3\text{-}NMe}$)‡, 31.9, 29.63, 29.62, 29.59, 29.48, 29.37, 29.33, 29.32 (CH_2 x8), 29.1 (C-4), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2923, 2854, 1661, 1464, 1340, 1178, 1097, 1002, 797, 721 cm^{-1}; HRMS Calcd for $[C_{16}H_{31}NO_2 + H]^+$: 270.2428, found 270.2429.

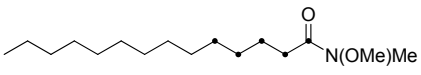


[1,2,3,4,5-$^{13}C_5$]-(*E*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (18*E) and [1,2,3,4,5-$^{13}C_5$]-(*Z*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (18*Z) [1,2,3-$^{13}C_3$]-*N*-Methoxy-*N*-methyl-dodecanamide **(16*)** (3.9 g, 16 mmol, 1.0 eq) was dissolved in anhydrous THF (120 mL) and cooled to 0 °C before addition of lithium aluminium hydride (4.0 M in THF) (2.4 mL, 9.5 mmol, 0.6 eq). The reaction was stirred for 45 minutes and was then cooled to −15 °C, before addition of sat. aq. $KHSO_4$ (100 mL) and diethylether (300 mL). The two-phase system was stirred vigorously for 30 minutes and was then dried with $MgSO_4$ followed by Na_2SO_4. The solids were filtered and washed with diethylether (200 mL). The eluate was concentrated *in vacuo* giving crude [1,2,3-$^{13}C_3$]-dodecanal (3.0 g, 16 mmol) as a clear oil which was used without further purification. Diethyl (*N*-Methoxy-*N*-methyl-carbamoylmethyl)phosphonate **(11)** (4.2 g, 17 mmol, 1.1 eq) was dissolved in anhydrous THF (80 mL) and cooled to 0 °C before addition of *n*-butyllithium (1.6 M in hexanes) (10.4 mL, 16.6 mmol, 1.05 eq). The reaction mixture was stirred for 10 minutes at 0 °C. The crude [1,2,3-$^{13}C_3$]-dodecanal was dissolved in anhydrous THF (20 mL) and added to the Horner-Wadsworth-Emmons reagent at 0 °C. The reaction was then stirred, going to room temperature and left stirring over night. The mixture was transferred to an extraction funnel with ether (50 mL) and washed with water (100 mL) and brine (100 mL). The water layers were extracted with ether (2 x 100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–15% EtOAc in petroleum ether) produced the title compounds **18*E** (3.1 g, 11 mmol, 70 %) and **18*Z** (0.31 g, 1.1 mmol, 7 %) in a combined yield of 77 % as clear oils. R_f **18*E** = 0.39; **18*Z** = 0.58 (15% EtOAc in petroleum ether). (***E*-isomer**, **18*E**) ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.98 (dm, 1 H, *J* = 153.8 Hz, H-3), 6.39 (ddm, 1 H, *J* = 161.1, 15.4 Hz, H-2), 3.70 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$), 2.23 (ddt, 2 H, *J* = 126.2, 7.0, 6.1 Hz, H-4), 1.60 – 1.20 (m, 18 H, H-5 to H-13), 0.88 (t, 3 H, *J* = 7.0 Hz, H-14); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 167.1 (dd, *J* = 67.1, 6.1 Hz, C=O), 148.0 (ddd, *J* = 71.6, 41.8, 2.1 Hz, C-3), 118.6 (dddd, *J* = 71.6, 67.1, 3.6, 1.5 Hz, C-2), 61.6 ($CH_{3\text{-}OMe}$), 32.5 (dddd, *J* = 41.8, 33.7, 6.1, 1.5 Hz, C-4), 32.3 ($CH_{3\text{-}NMe}$), 31.9 (CH_2), 29.6 – 29.0 (m, CH_2 x6), 28.3 (ddd, *J* = 33.7, 3.6, 2.1 Hz, C-5), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2924, 2854, 1618, 1583, 1464, 1368, 991 cm^{-1}; HRMS Calcd for $[C_{11}{}^{13}C_5H_{31}NO_2 + H]^+$: 275.2595, found 275.2595; (***Z*-isomer**, **18*Z**) ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.23 (dm, 1 H, *J* = 160.7 Hz, H-2), 6.12 (dm, 1 H, *J* = 152.0 Hz, H-3), 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.21 (s, 3 H, $CH_{3\text{-}NMe}$), 2.62 (dm, 2 H, *J* = 125.3 Hz, H-4), 1.59 – 1.20 (m, 18 H, H-5 to

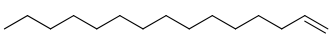
H-13), 0.88 (t, 3 H, *J* = 7.1 Hz, H-14); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 167.6 (dm, *J* = 67.1 Hz, C=O), 147.8 (dd, *J* = 69.9, 35.2 Hz, C-3), 117. 9 (dd, *J* = 69.9, 67.1 Hz, C-2), 61.4 ($CH_{3\text{-}OMe}$), 32.0 ($CH_{3\text{-}NMe}$)‡, 31.9 (CH_2), 30.2 – 28.4 (m, CH_2 x8), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2923, 2854, 1618, 1464, 1331, 1176, 1086, 999, 775 cm^{-1}; HRMS Calcd for $[C_{11}{}^{13}C_5H_{31}NO_2 + H]^+$: 275.2595, found 275.2595.



_N_-Methoxy-_N_-methyl-tetradecanamide (19) (*E*/*Z*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (**18E** and **18Z**) (1.1 g, 3.9 mmol, 1.0 eq) was dissolved in EtOAc (40 mL). The solution was purged with argon under stirring, before addition of palladium (10% on charcoal) (0.21 g, 0.20 mmol, 0.05 eq). The reaction mixture was then stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere over night. The palladium residue was removed by filtration over a Whatmann paper and rinsed with EtOAc (40 mL) followed by removal of the solvents under reduced preassure. Purification by column chromatography (5–15% EtOAc in petroleum ether) afforded the title compound as a clear oil (0.98 g, 3.6 mmol, 92%). R_f = 0.45 (20% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.18 (s, 3 H, $CH_{3\text{-}NMe}$), 2.41 (t, 2 H, *J* = 7.6 Hz, H-2), 1.63 (m, 2 H, H-3), 1.36 – 1.22 (m, 20 H, H-4 to H-13), 0.88 (t, 3 H, *J* = 6.8 Hz, H-14); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 174.5 (C=O), 61.2 ($CH_{3\text{-}OMe}$), 32.1 ($CH_{3\text{-}NMe}$), 31.9 (C-2), 29.64, 29.63, 29.62, 29.61 (x2), 29.5, 29.44, 29.41, 29.3 (CH_2 x9), 24.6 (C-3), 22.7 (CH_2), 14.1 (C-14).; IR (neat): 2923, 2853, 1668, 1464, 1413, 1383, 1176, 1001, 721 cm^{-1}; HRMS Calcd for $[C_{16}H_{33}NO_2 + H]^+$: 272.2584, found 272.2585.

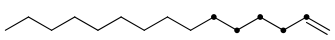


[1,2,3,4,5-$^{13}C_5$]-_N_-Methoxy-_N_-methyl-tetradecanamide (19*) [1,2,3,4,5-$^{13}C_5$]-(*E*/*Z*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (**18*E** and **18*Z**) (3.2 g, 11.7 mmol, 1.0 eq) was dissolved in EtOAc (100 mL). The solution was purged with argon under stirring, before addition of palladium (10% on charcoal) (0.62 g, 0.58 mmol, 0.05 eq). The reaction mixture was then stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere over night. The palladium residue was removed by filtration over a Whatmann paper and rinsed with EtOAc (100 mL) followed by removal of the solvents *in vacuo*. Purification by column chromatography (5–15% EtOAc in petroleum ether) afforded the title compound as a clear oil (3.0 g, 10.9 mmol, 93%). R_f = 0.38 (15% EtOAc in petroleum ether); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.18 (s, 3 H, $CH_{3\text{-}NMe}$), 2.41 (dm, 2 H, *J* = 128.4 Hz, H-2), 1.62 (dm, 2 H, *J* = 127.1 Hz, H-3), 1.46 – 1.12 (m, 20 H, H-4 to H-13), 0.88 (t, 3 H, *J* = 7.1 Hz, H-14); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 174.8 (dm, *J* = 51.5 Hz, C=O), 61.1 ($CH_{3\text{-}OMe}$), 32.1 ($CH_{3\text{-}NMe}$)‡, 31.9 (dd, *J* = 51.5, 35.6 Hz, C-2), 29.7 – 29.1 (m, CH_2 x9), 24.6 (m, C-3), 22.6 (CH_2), 14.1 (C-14).; IR (neat): 2922, 2853, 1628, 1458, 1370, 1175, 996, 721 cm^{-1}; HRMS Calcd for $[C_{11}{}^{13}C_5H_{33}NO_2 + H]^+$: 277.2751, found 277.2752.

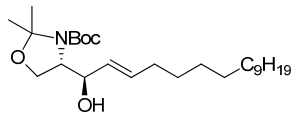


Pentadec-1-ene (20) *N*-Methoxy-*N*-methyl-tetradecanamide **(19)** (0.92 g, 3.4 mmol, 1.0 eq) was dissolved in anhydrous THF (34 mL) and $LiAlH_4$ (4 M in THF) (0.51 mL, 2.0 mmol, 0.6 eq) was added at 0 °C. The reaction was stirred for 45 minutes and then cooled to ca −15 °C before addition of sat. aq. $KHSO_4$ (15 mL) and diethylether (60 mL). The resulting two-phase mixture was stirred vigorously for 30 minutes and then dried with $MgSO_4$, and then Na_2SO_4. The solids were filtered and washed with diethylether (100 mL). The eluate was concentrated *in vacuo* giving crude tetradecanal (0.70 g, 3.3 mmol) as a clear oil which was used in the next step without further purification. Methyltriphenylphosphonium bromide (1.8 g, 5.1 mmol, 1.5 eq) was suspended in anhydrous THF (100 mL) and *n*-butyllithium (1.6 M in hexanes) (2.7 mL, 4.4 mmol, 1.3 eq) was added at 0 °C. The reaction was then stirred for 10 minutes at 0 °C. The crude tetradecanal, dissolved in anhydrous THF (10 mL), was then added to the ylide at 0 °C.

The reaction was stirred at room temperature over night and was then transferred to an extraction funnel with diethyl ether (50 mL). The reaction mixture was washed with water (100 mL x2) and brine (100 mL). The water phases were extracted with ether (100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (100 % petroleum ether) produced the title compound as a clear oil (0.56 g, 2.7 mmol, 79 %). R_f = 0.98 (100 % petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.79 (ddt, 1 H, *J* = 16.9, 10.2, 6.7 Hz, H-2), 4.98 (ddt, 1 H, *J* = 16.9, 2.1, 1.7 Hz, H-1$_Z$), 4.91 (ddt, 1 H, *J* = 10.2, 2.1, 1.2 Hz, H-1$_E$), 2.03 (q, 2 H, *J* = 6.9 Hz, H-3), 1.44 – 1.20 (m, 22 H, H-4 to H-14), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15)); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 139.1 (C-2), 114.1 (C-1), 33.9 (C-3), 32.1, 29.83, 29.82, 29.81, 29.79, 29.76, 29.65, 29.5, 29.3, 29.1, 22.8 (C-4 to C-14), 14.1 (C-15); IR (neat): 2922, 2854, 1467, 991, 909, 721 cm^{-1}. These data are in accordance with literature precedence and the ^{1}H-NMR is identical to the ^{13}C-decoupled ^{1}H-NMR of [2,3,4,5,6-$^{13}C_5$]-Pentadec-1-ene **(20*)**.

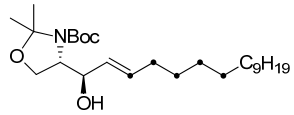


[2,3,4,5,6-$^{13}C_5$]-Pentadec-1-ene (20*) [1,2,3,4,5-$^{13}C_5$]-*N*-Methoxy-*N*-methyl-tetradecan-amide **(19*)** (1.6 g, 5.7 mmol, 1.0 eq) was dissolved in anhydrous THF (55 mL) and $LiAlH_4$ (4 M in THF) (0.86 mL, 3.4 mmol, 0.6 eq) was added at 0 °C. The reaction was stirred for 45 minutes and then cooled to ca −15 °C before addition of sat. aq. $KHSO_4$ (40 mL) and diethylether (100 mL). The resulting two-phase mixture was stirred vigorously for 30 minutes and then dried with $MgSO_4$, and then Na_2SO_4. The solids were filtered and washed with diethylether (100 mL). The eluate was concentrated *in vacuo* giving crude [1,2,3,4,5-$^{13}C_5$]-tetradecanal (1.2 g, 5.7 mmol) as a clear oil which was used in the next step without further purification. Methyltriphenylphosphonium bromide (3.1 g, 8.6 mmol, 1.5 eq) was suspended in anhydrous THF (150 mL) and *n*-butyllithium (1.6 M in hexanes) (4.7 mL, 7.4 mmol, 1.3 eq) was added at 0 °C. The reaction was then stirred for 10 minutes at 0 °C. The crude [1,2,3,4,5-$^{13}C_5$]-tetradecanal, dissolved in anhydrous THF (20 mL), was then added to the Horner-Wadsworth-Emmons reagent at 0 °C. The reaction was stirred at room temperature over night and was then transferred to an extraction funnel using 100 mL ether. The reaction mixture was washed with water (200 mL x2) and brine (200 mL). The water phases were extracted with ether (200 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (100 % petroleum ether) produced the title compound as a clear oil (1.2 g, 5.3 mmol, 93 %). R_f = 0.98 (100 % petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.81 (dm, 1 H, *J* = 150.3 Hz, H-2), 4.99 (dd, 1 H, *J* = 17.1, 6.5 Hz, H-1$_Z$), 4.92 (t, 1 H, *J* = 10.8 Hz, H-1$_E$), 2.03 (dm, 2 H, *J* = 125.4 Hz, H-3), 1.57 – 1.11 (m, 22 H, H-4 to H-14), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15); ^{1}H NMR (400 MHz, $CDCl_3$, ^{13}C-decoupled) Chemical shifts identical to the ^{1}H NMR presented for unlabeled Pentadec-1-ene **(20)**; ^{13}C NMR (101 MHz, $CDCl_3$) δ: 139.2 (dm, *J* = 42.1 Hz, C-2), 114.0 (dd, *J* = 69.1, 3.1 Hz, C-1), 33.9 (m, C-3), 32.0 (CH_2), 29.9 – 28.6 (m, CH_2 x9), 22.7 (CH_2), 14.1 (C-15) ; IR (neat): 2922, 2853, 1628, 1458, 1370, 1175, 1117, 996, 721 cm^{-1}.



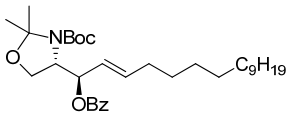
(*E*)-1,2-*O*,*N*-Isopropylidene-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (21E) and (*Z*)-1,2-*O*,*N*-Isopropylidene-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (21Z) (2*S*,3*R*)-2-Amino-*N*-(*tert*-butyloxycarbonyl)-1,3-dihydroxy-1,2-*O*,*N*-isopropylidene-4-pentene **(4)** (1.0 g, 4.0 mmol, 1.0 eq) and pentadec-1-ene **(20)** (1.7 g, 8.0 mmol, 2.0 eq) were dissolved in anhydrous DCM (4 mL) and flushed with argon before addition of Grubbs catalyst 2nd generation (67 mg, 79 µmol, 0.02 eq). The reaction was refluxed under an atmosphere of argon until TLC showed no change in reaction composition (~ 6 hours). The reaction was mixture was concentrated *in vacuo* and purified by column chromatography (0-10 % EtOAc in petroleum ether). The title compound was isolated as a viscous oil in a *E*/*Z*-ratio of 3.4:1 **(21E)** (0.84 g, 1.9 mmol, 48%) and **(21Z)** (0.25 g, 0.56 mmol, 14%).

(**E-isomer, 21E**): R_f = 0.19 (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: −26 (C = 0.25 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6, 363 °K) δ: 5.56 (dt, 1 H, *J* = 15.8, 6.5 Hz, H-5), 5.45 (ddd, 1 H, *J* = 15.8, 6.6, 1.1 Hz, H-4), 4.61 (bs, 1 H, OH), 4.03 (m, 1 H, H-3), 3.93 (bd, 1 H, *J* = 8.5 Hz, H-1_a), 3.83 (bt, 1 H, *J* = 7.3 Hz, H-1_b), 3.75 (m, 1 H, H-2), 1.98 (m, 2 H, H-6), 1.48 (s, 3 H, $CH_{3\text{-acetonide}}$), 1.43 (m, 12 H, $CH_{3\text{-acetonide}}$ and $CH_{3\text{-tBu-Boc}}$), 1.39 – 1.20 (m, 22 H, H-7 to H-17), 0.87 (t, 3 H, *J* = 6.6 Hz, H-18); ^{13}C NMR (100 MHz, DMSO-d_6, 363 °K) δ: 151.3 ($C{=}O_{Boc}$), 130.8 (C-5), 130.4 (C-4), 92.8 ($C_{q\text{-acetonide}}$), 78.7 ($C_{q\text{-Boc}}$), 71.4 (C-3), 63.7 (C-1), 61.0 (C-2), 31.2 (C-6), 30.8, 28.5 (x4), 28.4, 28.2, 28.12, 28.06, 27.7 (x3), 26.2, 21.5 (C-7 to C-17, $CH_{3\text{-tBu-Boc}}$ and $CH_{3\text{-acetonide}}$ x2), 13.2 (C-18); IR (neat): 3436, 2924, 2854, 1702, 1381, 1365, 1255, 1173, 1097, 848, 766 cm^{-1}; HRMS Calcd for $[C_{26}H_{49}NO_4 + H]^+$: 440.3734, found 440.3733. (**Z-isomer**, **21z**): R_f = 0.23 (10% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, DMSO-d_6, 363 °K) δ: 5.49 – 5.29 (m, 2 H, H-5 and H-4), 4.71 (bs, 1 H, OH), 3.99 (dd, 1 H, *J* = 8.5, 2.4 Hz, H-1_a), 3.82 (dd, 1 H, *J* = 8.5, 6.4 Hz, H-1_b), 3.75 (m, 1 H, H-3), 3.69 (m, 1 H, H-2), 2.07 (m, 2 H, H-6), 1.97 (m, 2 H, H-7), 1.46 (s, 3 H, $CH_{3\text{-acetonide}}$), 1.43 – 1.36 (m, 12 H, $CH_{3\text{-acetonide}}$ and $CH_{3\text{-tBu-Boc}}$), 1.35 – 1.19 (m, 20 H, H-8 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{13}C NMR (100 MHz, DMSO-d_6, 363 °K) δ: 151.4 ($C{=}O_{Boc}$), 131.2 (C-5), 126.7 (C-4), 92.8 ($C_{q\text{-acetonide}}$), 78.7 ($C_{q\text{-Boc}}$), 69.9 (C-2), 63.0 (C-1), 60.5 (C-3), 31.5 (C-6), 30.8, 28.5 (x4), 28.42, 28.35, 28.12, 28.01, 27.7 (x3), 27.5, 26.1, 21.5 (C-7 to C-17, $CH_{3\text{-tBu-Boc}}$ and $CH_{3\text{-acetonide}}$ x2), 13.2 (C-18); IR (neat): 3434, 2918, 2854, 1702, 1368, 1254, 1170, 1097, 848, 764 cm^{-1}; HRMS Calcd for $[C_{26}H_{49}NO_4 + H]$+: 440.3734, found 440.3734.

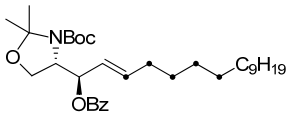


(*E*)-[5,6,7,8,9-$^{13}C_5$]-1,2-*O*,*N*-Isopropylidene-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (21*E) and (*Z*)-[5,6,7,8,9-$^{13}C_5$]-1,2-*O*,*N*-Isopropylidene-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (21*z) (2*S*,3*R*)-2-Amino-*N*-(*tert*-butyloxycarbonyl)-1,3-dihydroxy-1,2-*O*,*N*-isopropylidene-4-pentene **(4)** (3.6 g, 14 mmol, 3.0 eq) and [2,3,4,5,6-$^{13}C_5$]-pentadec-1-ene **(20*)** (1.0 g, 4.6 mmol, 1.0 eq) were dissolved in anhydrous DCM (4 mL) and flushed with argon before addition of Grubbs catalyst 2nd generation (79 mg, 93 µmol, 0.02 eq). The reaction was refluxed under a flow of argon until TLC showed no change in reaction composition (~ 6 hours). The reaction was mixture was concentrated *in vacuo* and purified by column chromatography (0–10 % EtOAc in petroleum ether). The title compound was isolated as a viscous oil in a *E*/*Z*-ratio of 3.5:1 **(21*E)** (1.3 g, 2.9 mmol, 63%) and **(21*z)** (0.38 g, 0.89 mmol, 18%). (**E-isomer**, **21*E**): R_f = 0.19 (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: −19 (C = 0.5 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6, 363 °K) δ: 5.55 (dm, 1 H, *J* = 152.0 Hz, H-5), 5.44 (m, 1 H, H-4), 4.60 (bd, 1 H, *J* = 5.4 Hz, OH), 4.05 (m, 1 H, H-3), 3.94 (dd, 1 H, *J* = 8.6, 2.0 Hz, H-1_a), 3.82 (dd, 1 H, *J* = 8.6, 6.1 Hz, H-1_b), 3.75 (td, 1 H, *J* = 6.1, 2.0 Hz, H-2), 1.98 (dm, 2 H, *J* 0 124.2 Hz, H-6), 1.56 – 1.06 (m, 37 H, $CH_{3\text{-tBu-Boc}}$, $CH_{3\text{-acetonide}}$ and H-7 to H-17), 0.87 (t, 3 H, *J* = 6.9 Hz, H-18); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.74 (dm, 1 H, *J* = 149.4 Hz, H-5), 5.45 (dd, 1 H, *J* = 15.4, 6.0 Hz, H-4), 4.39 – 3.74 (m, 5 H, H-3, H-2, H-1 and OH), 2.04 (dm, 2 H, *J* = 125.2 Hz, H-6), 1.72 – 1.01 (m, 37 H, $CH_{3\text{-tBu-Boc}}$, $CH_{3\text{-acetonide}}$ and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{1}H NMR (400 MHz, $CDCl_3$, ^{13}C-decoupled) δ: 5.74 (dt, 1 H, *J* = 15.4, 6.6 Hz, H-5), 5.45 (dd, 1 H, *J* = 15.4, 6.4 Hz, H-4), 4.39 – 3.74 (m, 5 H, H-3, H-2, H-1 and OH), 2.04 (q, 2 H, *J* = 7.0 Hz, H-6), 1.71 – 1.16 (m, 37 H, $CH_{3\text{-tBu-Boc}}$, $CH_{3\text{-acetonide}}$ and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{13}C NMR (100 MHz, DMSO-d_6, 363 °K) δ: 151.3 ($C{=}O_{Boc}$), 130.8 (d, *J* = 42.3 Hz, C-5), 130.4 (d, *J* = 73.4 Hz, C-4), 92.8 ($C_{q\text{-acetonide}}$), 78.4 ($C_{q\text{-Boc}}$), 71.4 (d, *J* = 5.2 Hz, C-3), 63.7 (C-1), 61.0 (d, *J* = 2.7 Hz, C-2), 31.9 – 30.5 (m, $C\text{-}6_{Sp}$ and $CH_{2\text{-Sp}}$), 29.7 – 26.1 ($CH_{2\text{-Sp}}$ x10, $CH_{3\text{-tBu-Boc}}$ and $CH_{3\text{-acetonide}}$ x2), 21.5 ($CH_{2\text{-Sp}}$), 13.2 ($C\text{-}18_{Sp}$); IR (neat): 3436, 2922, 2853, 1698, 1458, 1386, 1365, 1256, 1173, 1098, 965, 848, 766 cm^{-1}; HRMS Calcd for $[C_{21}{}^{13}C_5H_{49}NO_4 + H]^+$: 445.3902, found 445.3902. (**Z-isomer**, **21*z**): R_f = 0.23 (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: −10 (C = 0.5 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.58 (dm, 1 H, *J* = 152.1 Hz, H-5), 5.48 (dd, 1 H, *J* = 18.7, 9.3 Hz, H-4), 5.30 (bs, 1 H, OH), 4.65 (m, 1 H, H-3), 3.96 (dd, 1 H, *J* = 11.4, 3.7 Hz, H-1_a), 3.74 (dd, 1 H, *J* = 11.4, 3.7 Hz, H-1_b), 3.57 (m, 1 H, H-2), 2.13 (dm, 2 H, *J* = 122.5 Hz, H-6), 2.07 (dm, 2 H, *J* = 123.3 Hz, H-7), 1.57 – 1.03 (m, 35 H, $CH_{3\text{-acetonide}}$ (x2), $CH_{3\text{-tBu-Boc}}$ and H-8 to H-17), 0.84 (t, 3 H,

J = 6.8 Hz, H-18); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.2 ($C{=}O_{Boc}$), 134.5 (m, C-5), 128.5 (d, *J* = 69.5 Hz, C-4), 79.8 ($C_{q\text{-}Boc}$), 69.7 (C-2), 62.8 (C-1), 55.7 (C-3), 31.9 (C-6), 31.4 – 26.6 (m, CH_2 x9, $CH_{3\text{-}tBu\text{-}Boc}$ and $CH_{3\text{-}acetonide}$ x2), 22.7 (CH_2), 13.2 (C-18); HRMS Calcd for $[C_{21}{}^{13}C_5H_{49}NO_4 + H]^+$: 445.3902, found 445.3902.

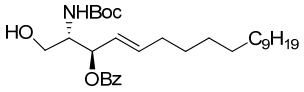


1,2-*O*,*N*-Isopropylidene-3-*O*-benzoyl-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (22) 1,2-*O*,*N*-Isopropylidene-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine **(21)** (0.59 g, 1.3 mmol, 1.0 eq) was dissolved in a 1:1 mixture of pyridine and DCM (10 mL). DMAP (16 mg, 0.13 mmol, 0.1 eq) was added followed by benzoyl chloride (0.23 mL, 2.0 mmol, 1.5 eq). The reaction was stirred over night and was then quenched with methanol (0.5 mL). The reaction was concentrated *in vacuo* and dissolved in EtOAc (50 mL). The organics was washed with 1 M HCl (50 mL), sat. aq. $NaHCO_3$ (50 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (1.5% EtOAc in petroleum ether) produced the title compound as a clear oil (0.61 g, 1.1 mmol, 84%). R_f = 0.82 (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: −29 (C = 0.66 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 363 °K) δ: 8.00 (dm, 1 H, *J* = 7.9 Hz, H_{arom}), 7.63 (m, 1 H, H_{arom}), 7.55 – 7.47 (m, 2 H, H_{arom}), 5.82 (bs, 1 H, H-3), 5.75 (dt, 1 H, *J* = 15.4, 6.5 Hz, H-5), 5.53 (ddd, 1 H, *J* = 15.4, 6.2, 1.4 Hz, H-4), 4.09 (m, 1 H, H-2), 4.06 – 3.97 (m, 2 H, H-1$_a$ and H-1$_b$), 2.01 (m, 2 H, H-6), 1.43 (s, 9 H, $CH_{3\text{-}tBu\text{-}Boc}$), 1.40 (s, 3 H, $CH_{3\text{-}acetonide}$), 1.36 – 1.17 (m, 25 H, $CH_{3\text{-}acetonide}$ and H-7 to H-17), 0.86 (t, 3 H, *J* = 6.6 Hz, H-18); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, 363 °K) δ: 164.5 ($C{=}O_{Bz}$), 134.4 (C-5), 132.7 (CH_{arom}), 129.7 ($C_{q\text{-}arom}$), 128.9, 128.1 (CH_{arom} x2), 125.2 (C-4), 93.2 ($C_{q\text{-}acetonide}$), 79.1 ($C_{q\text{-}Boc}$), 73.4 (C-3), 62.9 (C-1), 59.1 (C-2), 31.1, 30.8, 28.5 (x2), 28.4 (x2), 28.2, 28.4, 27.8 (x2), 27.6 (x2), 21.5 (C7-C17, $CH_{3\text{-}tBu\text{-}Boc}$ and $CH_{3\text{-}acetonide}$ x2), 13.3 (C-18); IR (neat): 2924, 2854, 1724, 1701, 1365, 1268, 1097, 1070, 855, 709 cm^{-1}; HRMS Calcd for $[C_{33}H_{53}NO_5 + Na]^+$: 566.3816, found 566.3814.

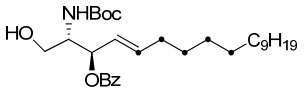


[5,6,7,8,9-$^{13}C_5$]-1,2-*O*,*N*-Isopropylidene-3-*O*-benzoyl-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (22*) [5,6,7,8,9-$^{13}C_5$]-1,2-*O*,*N*-Isopropylidene-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (1.1 g, 2.6 mmol, 1.0 eq) was dissolved in a 2:1 mixture of pyridine and DCM (20 mL). DMAP (16 mg, 0.13 mmol, 0.05 eq) was added followed by benzoyl chloride (0.45 mL, 3.9 mmol, 1.5 eq). The reaction was stirred over night and was then quenched with methanol (0.5 mL). The reaction was concentrated *in vacuo* and dissolved in EtOAc (50 mL). The organics was washed with 1 M HCl (50 mL), sat. aq. $NaHCO_3$ (50 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (1.5% EtOAc in petroleum ether) produced the title compound as a clear oil (1.1 g, 2.4 mmol, 92%). R_f = 0.29 (5% EtOAc in petroleum ether); $[\alpha]_D^{22}$: −30 (C = 0.5 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 363 °K) δ: 8.00 (d, 2 H, *J* = 7.6 Hz, H_{arom}), 7.64 (t, 1 H, *J* = 7.4 Hz, H_{arom}), 7.52 (t, 2 H, *J* = 7.6 Hz, H_{arom}), 5.82 (bs, 1 H, H-3), 5.75 (dm, 1 H, *J* = 149.2 Hz, H-5), 5.53 (m, 1 H, H-4), 4.15 – 3.97 (m, 3 H, H-2, H-1$_a$ and H-1$_b$), 2.04 (dm, 2 H, *J* = 126.1 Hz, H-6), 1.54 – 1.01 (m, 37 H, $CH_{3\text{-}tBu\text{-}Boc}$, $CH_{3\text{-}acetonide}$ x2 and H-7 to H-17), 0.86 (t, 3 H, *J* = 6.3 Hz, H-18); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, 363 °K) δ: 164.5 ($C{=}O_{Bz}$), 151.1 ($C{=}O_{Boc}$), 134.4 (d, *J* = 42.6 Hz, C-5), 132.8 (CH_{arom}), 129.7 ($C_{q\text{-}arom}$), 128.9, 128.2 (CH_{arom} x2), 125.2 (d, *J* = 72.2 Hz, C-4), 93.2 ($C_{q\text{-}acetonide}$), 79.2 ($C_{q\text{-}Boc}$), 73.4 (d, *J* = 5.6 Hz, C-3), 62.9 (C-1), 59.1 (C-2), 31.8 – 30.4 (m, C-6 and CH_2), 28.8 – 27.3 (m, CH_2 x9, $CH_{3\text{-}tBu\text{-}Boc}$ and $CH_{3\text{-}acetonide}$ x2), 21.6 (CH_2), 13.4 (C-18); The same sample in $CDCl_3$ at room temperature shows two rotamers: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10 (d, 2 H, *J* = 7.4 Hz, H_{arom}), 7.55 (t, 1 H, *J* = 7.4 Hz, H_{arom}), 7.44 (t, 2 H, *J* = 7.6 Hz, H_{arom}), 5.93 – 5.82 (m, 1 H, H-3), 5.82 (dm, 1 H, *J* = 149.8 Hz, H-5), 5.46 (m, 1 H, H-4), 4.25 – 4.10 (m, 1.5 H, H-2, H-1$_a$), 4.07 – 3.96 (m, 1.5 H, H-2, H-1$_b$), 2.03 (dm, 2 H, *J* = 125.7 Hz, H-6), 1.58 – 1.00 (m, 37 H,

$CH_{3\text{-tBu-Boc}}$, $CH_{3\text{-acetonide}}$ x2 and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.9 Hz, H-18); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.5, 165.4 ($C{=}O_{Bz}$ x2), 152.5, 151.7 ($C{=}O_{Boc}$ x2), 135.8 (d, *J* = 42.6 Hz, C-5) 135.7 (d, *J* = 42.6 Hz, C-5), 132.9, 132.8 (CH_{arom} x2), 130.5, 130.3 ($C_{q\text{-arom}}$ x2), 129.8 (CH_{arom}), 128.3 (CH_{arom}), 125.0 (d, *J* = 72.8 Hz, C-4), 94.6, 94.0 ($C_{q\text{-acetonide}}$ x2), 80.4, 80.2 ($C_{q\text{-Boc}}$ x2), 74.4 (d, *J* = 5.6 Hz, C-3), 74.2 (d, *J* = 5.6 Hz, C-3), 63.70, 63.66 (C-1 x2), 60.00, 59.97 (C-2 x2), 32.8 – 31.7 (m, C-6 and CH_2), 29.8 – 28.2 (m, CH_2 x9, $CH_{3\text{-tBu-Boc}}$ and $CH_{3\text{-acetonide}}$ x2), 22.7 (CH_2), 14.1 (C-18); HRMS Calcd for $[C_{28}{}^{13}C_5H_{53}NO_5 + Na]^+$: 571.3984, found 571.3982.

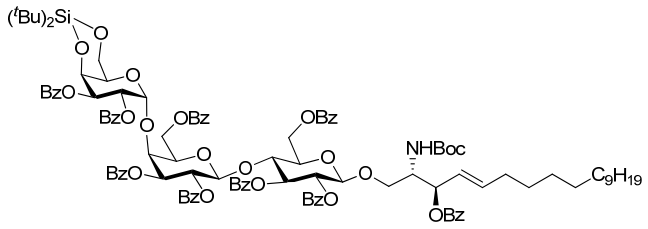


3-*O*-Benzoyl-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (23) 1,2-*O*,*N*-Isopropylidene-3-*O*-benzoyl-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (**22**) (0.50 g, 0.92 mmol, 1.0 eq) was dissolved in methanol:ethanol (1:1 15 mL) and *p*-toluenesulfonic acid (mono hydrate) (87 mg, 0.46 mmol, 0.5 eq) was added. The reaction was stirred at ambient temperature over night and was then quenched with triethylamine (0.32 mL, 2.3 mmol, 2.5 eq). The mixture was diluted with toluene (10 mL) and then concentrated *in vacuo*. The residue was dissolved in EtOAc (60 mL), washed with sat. aq. $NaHCO_3$ (60 mL) and brine (50 mL). The water layers were extracted with EtOAc (60 mL). The combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (10 % EtOAc in petroleum ether) produced the title compound as a clear waxy solid (0.25 g, 0.50 mmol, 54%; 88% based on recovered starting material). R_f = 0.07 (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +15 (C = 1.0 $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 (dm, 2 H, *J* = 7.5 Hz, H_{arom}), 7.57 (t, 1 H, *J* = 7.4 Hz, H_{arom}), 7.44 (t, 2 H, *J* = 7.7 Hz, H_{arom}), 5.87 (dt, 1 H, *J* = 14.9, 6.6 Hz, H-5), 5.60 (dd, 1 H, *J* = 14.9, 7.7 Hz, H-4), 5.53 (t, 1 H, *J* = 7.3 Hz, H-3), 5.12 (d, 1 H, *J* = 8.9 Hz, NH_{Boc}), 3.95 (m, 1 H, H-2), 3.76 – 3.67 (m, 2 H, H-1_a and H-1_b), 2.82 (bs, 1 H, OH), 2.05 (m, 2 H, H-6), 1.43 (s, 9 H, $CH_{3\text{-tBu-Boc}}$), 1.40 – 1.20 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.2 ($C{=}O_{Bz}$), 155.8 ($C{=}O_{Boc}$), 137.3 (C-5), 133.2 (CH_{arom}), 129.8 ($C_{q\text{-arom}}$), 129.7, 128.4 ($CH_{arom\ x}$2), 124.6 (C-4), 79.6 ($C_{q\text{-Boc}}$), 74.8 (C-3), 61.7 (C-1), 54.5 (C-2), 32.3 (C-6), 31.9, 29.62 (x3), 29.60, 29.5, 29.4, 29.3, 29.2, 28.9 (C_{Sp} x10), 28.3 ($CH_{3\text{-tBu-Boc}}$), 22.6 (C_{Sp}), 14.1 (C-18); IR (neat): 3372, 2924, 2854, 1715, 1268, 1171, 1111, 1070, 969, 710 cm^{-1}; HRMS Calcd for $[C_{30}H_{49}NO_5 + Na]^+$: 526.3503, found 526.3500.

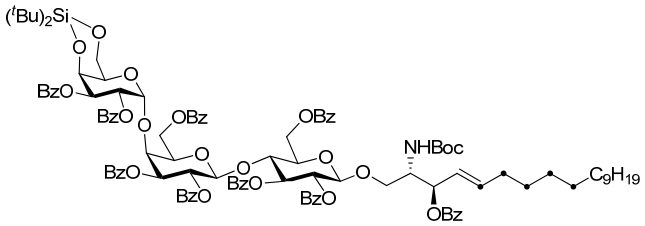


[5,6,7,8,9-$^{13}C_5$]-3-*O*-Benzoyl-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine (23*) Protected [5,6,7,8,9-$^{13}C_5$]-D-*erythro*-sphingosine **(22*)** (0.12 g, 0.22 mmol, 1.0 eq) was dissolved in methanol:ethanol (1:1, 10 mL) and *p*-toluenesulphonic acid (mono hydrate) (8.3 mg, 44 μmol, 0.2 eq) was added. The reaction was stirred at ambient temperature over night. The reaction mixture was transferred to an extraction funnel using EtOAc (60 mL) and washed with sat. aq. $NaHCO_3$:water 2:1 (60 mL) and brine (50 mL). The water layers were extracted with EtOAc (60 mL). The combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–10 % EtOAc in petroleum ether) produced the title compound as a clear oil that solidified upon standing (70 mg, 0.14 mmol, 63%; 83% based on recovered starting material). R_f = 0.07 (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +16 (C = 0.5 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 (dm, 2 H, *J* = 7.8 Hz, H_{arom}), 7.57 (tt, 1 H, *J* = 7.0, 1.5 Hz, H_{arom}), 7.45 (t, 2 H, *J* = 7.8 Hz, H_{arom}), 5.88 (dm, 1 H, *J* = 149.8 Hz, H-5), 5.60 (m, 1 H, H-4), 5.52 (m, 1 H, H-3), 5.08 (d, 1 H, *J* = 8.9 Hz, NH_{Boc}), 3.93 (m, 1 H, H-2), 3.76 – 3.67 (m, 2 H, H-1_a and H-1_b), 2.66 (bs, 1 H, OH), 2.08 (dm, 2 H, *J* = 125.5 Hz, H-6), 1.58 – 1.01 (m, 31 H, $CH_{3\text{-tBu-Boc}}$ and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{1}H NMR (400 MHz, $CDCl_3$, ^{13}C-decoupled is identical to ^{1}H NMR of compound **(23)**; ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.3 ($C{=}O_{Bz}$), 155.8 ($C{=}O_{Boc}$), 137.4 (d, *J* = 42.5 Hz, C-5), 133.3 (CH_{arom}), 129.80 ($C_{q\text{-arom}}$), 129.75, 128.4 ($CH_{arom\ x}$2), 124.6 (d, *J* = 71.5 Hz, C-4), 79.7 ($C_{q\text{-Boc}}$), 74.9 (d, *J* = 5.4 Hz, C-3), 61.9 (C-1), 54.6 (C-2), 33.0 – 31.6 (C-6 and CH_2), 29.8 – 28.1 (CH_2 x9 and $CH_{3\text{-tBu-Boc}}$), 22.7

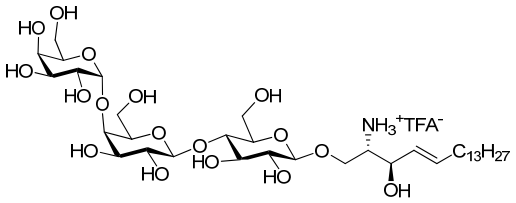
(CH_2), 14.1 (C-18); IR (neat): 3372, 2922, 2853, 1696, 1505, 1452, 1267, 1169, 1111, 1070, 1026, 966, 710 cm^{-1}; HRMS Calcd for $[C_{25}{}^{13}C_5H_{49}NO_5 + Na]^+$: 531,3671, found 531,3667.



3-*O*-Benzoyl-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine-1-yl 2,3,6-tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl-*α*-D-galactopyranosyl)-*α*-D-galactopyranosyl]-*β*-D-glucopyranoside (25) Globotriaosyl imidate donor **(24)** (0.54 g, 0.33 mmol, 1.2 eq) and sphingosine acceptor **(23)** (0.14 mg, 0.27 mmol, 1.0 eq) were co-evaporated twice in toluene (5 mL) and then dissolved in anhydrous DCM (3 mL). Activated molsieves (3Å) were added and the mixture was stirred for one hour at ambient temperature and then cooled to 0 °C, before addition of $BF_3{\cdot}Et_2O$ (48% in Et_2O) (38 µL, 0.30 mmol, 1.1 eq). The reaction was stirred until TLC showed complete conversion of the sphingosine acceptor (~1.5 hours). The reaction mixture was then transferred to an extraction funnel with EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (30 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (12% ether, 10% DCM in petroleum ether) produced the title compound as an amorphous solid (0.32 g, 0.16 mmol, 60%). R_f = 0.54 (30% ether, 20% DCM in petroleum ether); $[\alpha]_D^{22}$: +31 (C = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.19 (dm, 2 H, *J* = 7.2 Hz, H_{arom}), 8.07 – 8.00 (m, 4 H, H_{arom}), 7.96 (m, 2 H, H_{arom}), 7.92 – 7.85 (m, 6 H, H_{arom}), 7.68 (dm, 2 H, *J* = 7.2 Hz, H_{arom}), 7.58 – 7.41 (m, 9 H, H_{arom}), 7.40 – 7.18 (m, 18 H, H_{arom}), 7.11 (tm, 2 H, *J* = 7.7 Hz, H_{arom}), 5.78 (t, 1 H, *J* = 9.3 Hz, H-3), 5.72 (dd, 1 H, *J* = 10.7, 3.7 Hz, H-2′′), 5.66 (m, 1 H, H-5_{Sp}), 5.60 (dd, 1 H, *J* = 10.8, 7.8 Hz, H-2′), 5.50 (dd, 1 H, *J* = 10.7, 3.0 Hz, H-3′′), 5.47 – 5.32 (m, 4 H, H-3_{Sp}, H-2, H-4_{Sp} and H-1′′), 5.25 (dd, 1 H, *J* = 10.8, 2.1 Hz, H-3′), 5.10 (d, 1 H, *J* = 2.9 Hz, H-4′′), 4.81 – 4.74 (m, 2 H, H-1′ and H_{NBoc}), 4.66 (d, 1 H, *J* = 7.8 Hz, H-1), 4.55 (d, 1 H, *J* = 11.9 Hz, H-6_a′′), 4.46 (d, 1 H, *J* = 11.9 Hz, H-6_b′′), 4.39 – 4.32 (m, 3 H, H-5′′, H-6_a and H-6_b), 4.12 (t, 1 H, *J* = 9.3 Hz, H-4), 4.08 (bs, 1 H, H-4′), 4.07 – 4.00 (m, 2 H, H-$1_{a\text{-}Sp}$ and H-2_{Sp}), 3.97 (dd, 1 H, *J* = 10.9, 5.3 Hz, H-6_a′), 3.81 – 3.72 (m, 2 H, H-5 and H-6_b′), 3.59 (m, 1 H, H-$1_{b\text{-}Sp}$), 3.53 (m, 1 H, H-5′), 1.88 (m, 2 H, H-6_{Sp}), 1.33 (s, 9 H, $CH_{3\text{-}tBu\text{-}Boc}$), 1.30 – 1.11 (m, 22 H, H-7_{Sp} to H-17_{Sp}), 1.06 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.00 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.87 (t, 3 H, *J* = 6.8 Hz, H-18_{Sp}); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.2, 165.9, 165.7, 165.6, 165.2, 164.98, 164.95, 164.8, 164.7 ($C{=}O_{Bz}$ x9), 155.2 ($C{=}O_{Boc}$), 137.2 (C-5_{Sp}), 133.4, 133.13, 133.10, 132.97, 132.94, 132.87, 132.7 (CH_{arom} x7), 130.2 ($C_{q\text{-}arom}$), 130.1, 130.0 (CH_{arom} x2), 129.9 ($C_{q\text{-}arom}$), 129.8, 129.64, 129.59, 129.58, 129.5 (CH_{arom} x5), 129.4 ($C_{q\text{-}arom}$), 129.3 (CH_{arom}), 129.2, 129.0, 128.6, 128.50, 128.47 ($C_{q\text{-}arom}$ x5), 128.45, 128.39, 128.35, 128.29, 128.28, 128.16, 128.12, 128.07 (CH_{arom} x8), 124.4 (C-4_{Sp}), 101.3 (C-1′), 100.9 (C-1), 98.68 (C-1′′), 79.3 ($C_{q\text{-}Boc}$), 76.6 (C-4), 76.3 (C-4′), 74.3 (C-3_{Sp}), 73.02 (C-5), 72.94 (C-3), 72.8 (C-3′), 72.6 (C-5′), 71.9 (C-2), 71.2 (C-3′′), 71.0 (C-4′′), 69.7 (C-2′), 69.5 (C-2′′), 68.3 (C-5′′), 67.8 (C-1_{Sp}), 66.9 (C-6′), 62.3 (C-6), 60.5 (C-6′′), 52.3 (C-2_{Sp}), 32.2 (C-6_{Sp}), 31.9, 29.62 (x3), 29.61, 29.5, 29.4, 29.3, 29.2, 28.7 ($CH_{2\text{-}Sp}$ x10), 28.2 ($CH_{3\text{-}tBu\text{-}Boc}$), 27.5, 27.2 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2 ($C_{q\text{-}tBu\text{-}Si}$), 22.6 ($CH_{2\text{-}Sp}$), 20.7 ($C_{q\text{-}tBu\text{-}Si}$), 14.1 (C-$18_{Sp}$); IR (neat): 3070, 2926, 2856, 1722, 1451, 1267, 1095, 1070, 1028, 708 cm^{-1}; HRMS Calcd for $[C_{112}H_{127}NO_{28}Si + Na]^+$: 1984.8206, found 1984.8204.

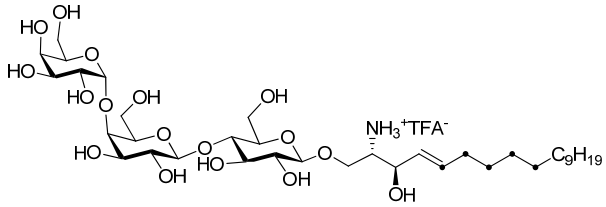


[5,6,7,8,9-$^{13}C_5$]-(E)-(2*R*,3*S*)-2-Amino-1,3-dihydroxy-octadec-4-ene-1-yl 2,3,6-tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl-*α*-D-galactopyranosyl)-*α*-D-galactopyranosyl]-*β*-D-glucopyranoside (25*) Globotriaosyl imidate donor **(24)** (0.16 g, 96 µmol, 1.2 eq) and $^{13}C_5$-sphingosine acceptor **(23*)** (41 mg, 80 µmol, 1.0 eq) were co-evaporated two times in toluene (5 mL) and then dissolved in anhydrous DCM (2 mL) Activated molsieves (3Å) were added and the mixture was stirred for one hour at ambient temperature and then cooled to 0 ºC, before addition of $BF_3{\cdot}OEt_2$ (48% in Et_2O) (23 µL, 88 µmol, 1.1 eq). The reaction was stirred until TLC showed complete conversion of the $^{13}C_5$-sphingosine acceptor (~2 hours). The reaction mixture was then transferred to an extraction funnel with EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (30 mL). The aqueous layers were then extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (12% ether, 10% DCM in petroleum ether) produced the title compound as an amorphous solid (87 mg, 44 µmol, 55%). R_f = 0.54 (30% ether, 20% DCM in petroleum ether); $[\alpha]_D^{22}$: +30 (C = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17 (dm, 2 H, *J* = 7.6 Hz, H_{arom}), 8.06 – 8.00 (m, 4 H, H_{arom}), 7.95 (dm, 2 H, *J* = 7.7 Hz, H_{arom}), 7.92 – 7.84 (m, 6 H, H_{arom}), 7.67 (dm, 2 H, *J* = 7.6 Hz, H_{arom}), 7.55 (m, 2 H, H_{arom}), 7.53 – 7.42 (m, 7 H, H_{arom}), 7.40 – 7.27 (m, 16 H, H_{arom}), 7.21 (tm, 2 H, *J* = 7.6 Hz, H_{arom}), 7.11 (tm, 2 H, *J* = 7.8 Hz, H_{arom}), 5.77 (t, 1 H, *J* = 9.3 Hz, H-3), 5.71 (dd, 1 H, *J* = 10.7, 3.7 Hz, H-2″), 5.67 (dm, 1 H, *J* = 151.2 Hz, H-5_{Sp}), 5.59 (dd, 1 H, *J* = 10.8, 7.8 Hz, H-2′), 5.49 (dd, 1 H, *J* = 10.7, 3.0 Hz, H-3″), 5.47 – 5.31 (m, 4 H, H-3_{Sp}, H-2, H-4_{Sp} and H-1″), 5.24 (dd, 1 H, *J* = 10.9, 2.1 Hz, H-3′), 5.10 (d, 1 H, *J* = 3.0 Hz, H-4″), 4.80 – 4.73 (m, 2 H, H-1′ and H_{NBoc}), 4.65 (d, 1 H, *J* = 7.8 Hz, H-1), 4.54 (d, 1 H, *J* = 12.0 Hz, H-6_a″), 4.44 (d, 1 H, *J* = 12.0 Hz, H-6_b″), 4.38 – 4.30 (m, 3 H, H-5″, H-6_a and H-6_b), 4.12 (t, 1 H, *J* = 9.4 Hz, H-4), 4.07 (d, 1 H, *J* = 1.5 Hz, H-4′), 4.06 – 3.99 (m, 2 H, H-$1_{a\text{-}Sp}$ and H-2_{Sp}), 3.97 (dd, 1 H, *J* = 10.9, 5.4 Hz, H-6_a′), 3.81 – 3.71 (m, 2 H, H-5 and H-6_b′), 3.58 (m, 1 H, H-$1_{b\text{-}Sp}$), 3.51 (dd, 1 H, *J* = 13.9, 6.9 Hz, H-5′), 1.87 (dm, 2 H, *J* = 124.6 Hz, H-6_{Sp}), 1.40 – 1.14 (m, 31 H, H-7_{Sp} to H-$17_{Sp,}$ and $CH_{3\text{-}tBu\text{-}Boc}$), 1.05 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.00 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.87 (t, 3 H, *J* = 6.8 Hz, H-18_{Sp}); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.2, 166.0, 165.7, 165.6, 165.2, 165.00, 164.98, 164.79, 164.75 (C=O_{Bz} x9), 155.2 (C=O_{Boc}), 137.2 (d, *J* = 42.4 Hz, C-5_{Sp}), 133.4, 133.2, 133.1, 133.00, 132.99, 132.96, 132.89, 132.7, 130.2, 130.1, 130.00, 129.95, 129.8, 129.67, 129.62, 129.60, 129.50, 129.48, 129.4, 129.2, 129.0, 128.6, 128.53, 128.50, 128.48, 128.41, 128.37, 128.32, 128.30, 128.18, 128.14, 128.09 (CH_{arom} and $C_{q\text{-}arom}$ x32), 124.4 (d, *J* = 71.2 Hz, C-4_{Sp}), 101.3 (C-1′), 100.9 (C-1), 98.7 (C-1″), 79.4 ($C_{q\text{-}Boc}$), 76.6 (C-4), 76.3 (C-4′), 74.3 (d, *J* = 5.4 Hz, C-3_{Sp}), 73.04 (C-5), 72.97 (C-3), 72.8 (C-3′), 72.7 (C-5′), 71.9 (C-2), 71.2 (C-3″), 71.0 (C-4″), 69.7 (C-2′), 69.6 (C-2″), 68.3 (C-5″), 67.8 (C-1_{Sp}), 66.9 (C-6′), 62.3 (C-6), 60.5 (C-6″), 52.3 (d, *J* = 2.4 Hz, C-2_{Sp}), 32.2 (m, C-6_{Sp}), 31.9 ($CH_{2\text{-}Sp}$), 29.8 – 28.1 (m, $CH_{2\text{-}Sp}$ x9 and $CH_{3\text{-}tBu\text{-}Boc}$), 27.5, 27.2 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2 ($C_{q\text{-}tBu\text{-}Si}$), 22.7 ($C_{Sp}$), 20.7 ($C_{q\text{-}tBu\text{-}Si}$), 14.1 (C-$18_{Sp}$); IR (neat): 3070, 2925, 2853, 1718, 1452, 1266, 1094, 1069, 706 cm^{-1}; HRMS Calcd for $[C_{107}{}^{13}C_5H_{127}O_{28}Si + Na]^+$: 1989.8374, found 1989.8370.



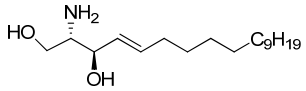
Globotriaosylsphingosine (26a-c) Protected globotriaosylsphingosine (**25**) (0.2 g, 0.10 mmol, 1.0 eq) was dissolved in THF:pyridine 4:1 (20 mL) and hydrogen fluoride (70% HF in pyridine) (53 µL, 0.26 mmol, ca. 20 eq, based on HF) was added. The reaction was stirred at room temperature until TLC showed full conversion to a lower running spot (~ 4 hours). The reaction was then concentrated *in vacuo*, re-dissolved in EtOAc (50 mL) and washed with 1 M HCl (50 mL), sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL). The water phases were extracted with EtOAc (50 mL) and the combined organics was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude mixture was then dissolved in methanol (20 mL) and sodium methoxide (30% in MeOH) (14.1 µL, 0.102 mmol, 1.0 eq) was added. The reaction was stirred over night at ambient temperature and the progression of the reaction was followed by HPLC-MS. Aqueous potassium hydroxide (0.5 M) (4.1 mL, 2.04 mmol, 20 eq) was added and the reaction was left stirring over night at ambient temperature. The reaction was then quenched with AcOH (0.58 mL, 100 eq) and concentrated *in vacuo*. The crude reaction mixture was coevaporated in toluene and put on ice-bath before addition of trifluoroacetic acid (5 mL). The reaction mixture was completely dissolved by the TFA after one minute and was then stirred for another minute at 0 °C. The solution was then transferred to a round bottom flask containing toluene (50 mL) and concentrated to about 10 mL *in vacuo*. The coevaporation was repeted two times with toluene (40 mL), before concentration to dryness. The completion of the reaction was confirmed by HPLC–MS. The residue was then roughly purified over a short silica column and eluted with MeOH/DCM 1:9 followed by H_2O/MeOH/DCM 3:27:70 (TLC visualised with ninhydrine spray). Purification by HPLC–MS (40–48% B, following the general procedure for HPLC–MS purifications) produced Globotriaosylsphingosine **(26a)** (32 mg, 40 µmol, 39 %), its nor-homologue **(26b)** (10 mg, 13 µmol, 13 %) and di-nor-homologue **(26c)** (1 mg, 1.3 µmol, 1 %) as their respective TFA adducts. **Globotriaosylsphingosine (26a)** $[\alpha]_D^{22}$: +34 (C = 0.5 MeOH); ^{1}H NMR (600 MHz, MeOD-d_4) δ: 5.87 (dtd, 1 H, *J* = 15.4, 6.8, 1.3 Hz, H-5_{Sp}), 5.49 (ddt, 1 H, *J* = 15.4, 6.8, 1.5 Hz, H-4_{Sp}), 4.94 (d, 1 H, *J* = 3.9 Hz, H-1″), 4.40 (d, 1 H, *J* = 6.9 Hz, H-1′), 4.37 (d, 1 H, *J* = 7.8 Hz, H-1), 4.32 (ddd, 1 H, *J* = 6.8, 4.7, 1.3 Hz, H-3_{Sp}), 4.25 (ddd, 1 H, *J* = 7.1, 5.2, 1.3 Hz, H-5″), 4.01 – 3.96 (m, 2 H, H-4′ and H-$1_{a\text{-}Sp}$), 3.94 (dd, 1 H, *J* = 11.9, 2.6 Hz, H-6_a), 3.93 – 3.91 (m, 2 H, H-4″ and H-$1_{b\text{-}Sp}$), 3.89 (dd, 1 H, *J* = 7.7, 4.1 Hz, H-6_b), 3.88 – 3.81 (m, 3 H, H-6_a′, H-6_b′ and H-2″), 3.77 (dd, 1 H, *J* = 10.2, 3.2 Hz, H-3″), 3.74 (dd, 1 H, *J* =11.1, 7.1 Hz, H-6_a″), 3.71 – 3.66 (m, 2 H, H-5′ and H-6_b″), 3.58 – 3.51 (m, 4 H, H-4, H-3, H-3′ and H-2′), 3.47 (m, 1 H, H-5), 3.40 (ddd, 1 H, *J* = 8.5, 4.7, 3.6 Hz, H-2_{Sp}), 3.30 (t, 1 H, *J* = 7.7 Hz, H-2), 2.10 (q, 2 H, *J* = 7.0 Hz, H-6_{Sp}), 1.42 (m, 2 H, H-7_{Sp}), 1.36 – 1.25 (m, 20 H, H-8_{Sp} to H-17_{Sp}), 0.90 (t, 3 H, *J* = 7.0 Hz, H-18_{Sp}); ^{13}C NMR (151 MHz, MeOD-d_4) δ: 136.8 (C-5_{Sp}), 128.3 (C-4_{Sp}), 105.4 (C-1′), 103.7 (C-1), 102.7 (C-1″), 80.8 (C-4), 79.8 (C-4′), 76.6 (C-5′ and C-5), 76.3 (C-2′), 74.7 (C-3), 74.6 (C-2), 72.8 (C-5″), 72.6 (C-3′), 71.3 (C-4″), 71.0 (C-3″), 70.8 (C-3_{Sp}), 70.5 (C-2″), 67.1 (C-1_{Sp}), 62.7 (C-6″), 61.6 (C-6), 61.5 (C-6′), 56.7 (C-2_{Sp}), 33.4 (C-6_{Sp}), 33.1, 30.79 (x3), 30.76, 30.74, 30.6, 30.5, 30.4, 30.2, 23.7 ($CH_{2\text{-}Sp}$ x11), 14.4 (C-18_{Sp}); IR (neat): 3344 bs, 2925, 2855, 1674, 1202, 1134, 1067, 1027, 974, 801, 721cm^{-1}; HRMS Calcd for $[C_{36}H_{67}NO_{17} + H]^+$: 786.4482 Found 786.4485. **Nor-globotriaosylsphingosine** (-14) **(26b)** ^{1}H NMR (600 MHz, MeOD-d_4) δ: 5.87 (dtd, 1 H, *J* = 15.4, 6.8, 1.3 Hz, H-5_{Sp}), 5.49 (ddt, 1 H, *J* = 15.4, 6.8, 1.5 Hz, H-4_{Sp}), 4.94 (d, 1 H, *J* = 3.9 Hz, H-1″), 4.40 (d, 1 H, *J* = 6.9 Hz, H-1′), 4.36 (d, 1 H, *J* = 7.8 Hz, H-1), 4.31 (ddd, 1 H, *J* = 6.8, 4.7, 1.3 Hz, H-3_{Sp}), 4.25 (ddd, 1 H, *J* = 7.1, 5.2, 1.3 Hz, H-5″), 4.01 – 3.96 (m, 2 H, H-4′ and H-$1_{a\text{-}Sp}$), 3.94 (dd, 1 H, *J* = 11.9, 2.6 Hz, H-6_a′), 3.93 – 3.91 (m, 2 H, H-4″ and H-$1_{b\text{-}Sp}$), 3.89 (dd, 1 H, *J* = 7.7,

4.1 Hz, H-6_b), 3.88 – 3.81 (m, 3 H, H-6_a′, H-6_b′ and H-2″), 3.77 (dd, 1 H, *J* = 10.2, 3.2 Hz, H-3″), 3.74 (dd, 1 H, *J* =11.1, 7.1 Hz, H-6_a″), 3.71 – 3.66 (m, 2 H, H-5′ and H-6_b″), 3.58 – 3.51 (m, 4 H, H-4, H-3, H-3′ and H-2′), 3.47 (m, 1 H, H-5), 3.40 (ddd, 1 H, *J* = 8.5, 4.7, 3.6 Hz, H-2_{Sp}), 3.30 (t, 1 H, *J* = 7.7 Hz, H-2), 2.10 (q, 2 H, *J* = 7.0 Hz, H-6_{Sp}), 1.42 (m, 2 H, H-7_{Sp}), 1.36 – 1.25 (m, 18 H, H-8_{Sp} to H-16_{Sp}), 0.90 (t, 3 H, *J* = 7.0 Hz, H-17_{Sp}); ^{13}C NMR (151 MHz, MeOD-d_4) δ: 136.8 (C-5_{Sp}), 128.3 (C-4_{Sp}), 105.4 (C-1′), 103.7 (C-1), 102.7 (C-1″), 80.8 (C-4), 79.8 (C-4′), 76.6 (C-5′ and C-5), 76.3 (C-2′), 74.7 (C-3), 74.6 (C-2), 72.8 (C-5″), 72.6 (C-3′), 71.3 (C-4″), 71.0 (C-3″), 70.8 (C-3_{Sp}), 70.5 (C-2″), 67.1 (C-1_{Sp}), 62.7 (C-6″), 61.6 (C-6), 61.5 (C-6′), 56.7 (C-2_{Sp}), 33.4 (C-6_{Sp}), 33.1, 30.80 (x2), 30.76, 30.75, 30.6, 30.5, 30.4, 30.2, 23.7 ($CH_{2\text{-}Sp}$ x10), 14.4 (C-17_{Sp}); IR (neat): 3344 bs, 2925, 2855, 1674, 1202, 1134, 1067, 1027, 974, 801, 721cm^{-1}; HRMS Calcd for $[C_{35}H_{65}NO_{17} + H]^+$: 772.4325, found 772.4329. **Di-nor-globotriaosylsphingosine** (-28) **(26c)** ^{1}H NMR (600 MHz, MeOD-d_4) δ: 5.87 (dtd, 1 H, *J* = 15.4, 6.8, 1.3 Hz, H-5_{Sp}), 5.49 (ddt, 1 H, *J* = 15.4, 6.8, 1.5 Hz, H-4_{Sp}), 4.94 (d, 1 H, *J* = 3.9 Hz, H-1″), 4.40 (d, 1 H, *J* = 6.9 Hz, H-1′), 4.36 (d, 1 H, *J* = 7.8 Hz, H-1), 4.31 (ddd, 1 H, *J* = 6.8, 4.7, 1.3 Hz, H-3_{Sp}), 4.25 (ddd, 1 H, *J* = 7.1, 5.2, 1.3 Hz, H-5″), 4.01 – 3.96 (m, 2 H, H-4′ and H-$1_{a\text{-}Sp}$), 3.94 (dd, 1 H, *J* = 11.9, 2.6 Hz, H-6_a), 3.93 – 3.91 (m, 2 H, H-4″ and H-$1_{b\text{-}Sp}$), 3.89 (dd, 1 H, *J* = 7.7, 4.1 Hz, H-6_b), 3.88 – 3.81 (m, 3 H, H-6_a′, H-6_b′ and H-2″), 3.77 (dd, 1 H, *J* = 10.2, 3.2 Hz, H-3″), 3.74 (dd, 1 H, *J* =11.1, 7.1 Hz, H-6_a″), 3.71 – 3.66 (m, 2 H, H-5′ and H-6_b″), 3.58 – 3.51 (m, 4 H, H-4, H-3, H-3′ and H-2′), 3.47 (m, 1 H, H-5), 3.40 (ddd, 1 H, *J* = 8.5, 4.7, 3.6 Hz, H-2_{Sp}), 3.30 (t, 1 H, *J* = 7.7 Hz, H-2), 2.10 (q, 2 H, *J* = 7.0 Hz, H-6_{Sp}), 1.42 (m, 2 H, H-7_{Sp}), 1.36 – 1.25 (m, 16 H, H-8_{Sp} to H-15_{Sp}), 0.90 (t, 3 H, *J* = 7.0 Hz, H-16_{Sp}); ^{13}C NMR (151 MHz, MeOD-d_4) δ: 136.8 (C-5_{Sp}), 128.3 (C-4_{Sp}), 105.4 (C-1′), 103.7 (C-1), 102.7 (C-1″), 80.8 (C-4), 79.8 (C-4′), 76.6 (C-5′ and C-5), 76.3 (C-2′), 74.7 (C-3), 74.6 (C-2), 72.8 (C-5″), 72.6 (C-3′), 71.3 (C-4″), 71.0 (C-3″), 70.8 (C-3_{Sp}), 70.5 (C-2″), 67.1 (C-1_{Sp}), 62.7 (C-6″), 61.6 (C-6), 61.5 (C-6′), 56.7 (C-2_{Sp}), 33.4 (C-6_{Sp}), 33.1, 30.81, 30.77, 30.76, 30.6, 30.5, 30.4, 30.2, 23.7 ($CH_{2\text{-}Sp}$ x9), 14.4 (C-16_{Sp}); HRMS Calcd for $[C_{34}H_{63}NO_{17} + H]^+$: 758.4173, found 758.4169.



Globotriaosyl-[5,6,7,8,9-$^{13}C_5$]-sphingosine (26*a-c) Protected globotriaosyl-[5,6,7,8,9-$^{13}C_5$]-sphingosine (**25***) (88 mg, 0.45 µmol, 1.0 eq) was dissolved in THF:pyridine 4:1 (10 mL) and hydrogen fluoride (70% HF in pyridine) (24 µL, 0.11 mmol, ca. 20 eq, based on HF) was added. The reaction was stirred at room temperature until TLC showed full conversion to a lower running spot (~ 4 hours). The reaction was then concentrated *in vacuo*, re-dissolved in EtOAc (50 mL) and washed with 1 M HCl (50 mL), sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous phases were extracted with EtOAc (50 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude mixture was then dissolved in methanol (8 mL) and sodium methoxide (30% in MeOH) (6.2 µL, 0.45 µmol, 1.0 eq) was added. The reaction was stirred over night at ambient temperature. The progression of the reaction was monitored by HPLC–MS. Aqueous potassium hydroxide (0.5 M) (1.8 mL, 0.89 mmol, 20 eq) was added and the reaction was left stirring over night at ambient temperature. The reaction was then quenched with AcOH (0.25 mL, 100 eq) and concentrated *in vacuo*. The crude reaction mixture was coevaporated in toluene and put on ice-bath before addition of trifluoroacetic acid (3 mL). The reaction mixture was completely dissolved by TFA in about one minute and was then stirred for an additional minute at 0 °C. The solution was then transferred to a round bottom flask containing toluene (50 mL) and concentrated to about 10 mL *in vacuo*. The coevaporation was repeated two times with toluene (40 mL), before concentration to dryness.

The completion of the reaction was controlled by HPLC–MS. The residue was then filtered over a small silica column and eluted with MeOH/DCM 1:9 and then H_2O/MeOH/DCM 3:27:70 (TLC visualised with ninhydrine spray). Purification by HPLC–MS (40–48% B, following the general procedure for HPLC–MS purifications) produced globotriaosylsphingosine **(26*a)** (17 mg, 21 µmol, 48%), its nor-homologue **(26*b)** (3.7 mg, 4.8 µmol, 11%) and di-nor-homologue **(26*c)** (0.4 mg, 1.0 µmol, 1 %) as their respective TFA adducts. **Globotriaosyl-[5,6,7,8,9-$^{13}C_5$]-sphingosine (26*a)** $[\alpha]_D^{22}$: +33.0 (C = 0.20 MeOH); ^{1}H NMR (600 MHz, MeOD-d_4) δ: 5.85 (dm, 1 H, *J* = 150.2 Hz, H-5$_{Sp}$), 5.47 (ddt, 1 H, *J* = 15.4, 6.4 Hz, H-4$_{Sp}$), 4.94 (d, 1 H, *J* = 3.8 Hz, H-1′′), 4.39 (d, 1 H, *J* = 7.1 Hz, H-1′), 4.36 (d, 1 H, *J* = 7.8 Hz, H-1), 4.31 (ddd, 1 H, *J* = 6.4, 4.8 Hz, H-3$_{Sp}$), 4.25 (ddd, 1 H, *J* = 6.8, 5.2, 1.3 Hz, H-5′′), 4.01 – 3.96 (m, 2 H, H-4′ and H-1$_{a\text{-}Sp}$), 3.94 (dd, 1 H, *J* = 12.0, 2.4 Hz, H-6$_a$), 3.92 – 3.90 (m, 2 H, H-4′′ and H-1$_{b\text{-}Sp}$), 3.89 (dd, 1 H, *J* = 7.7, 4.0 Hz, H-6$_b$), 3.88 – 3.79 (m, 3 H, H-6$_a$′, H-6$_b$′ and H-2′′), 3.77 (dd, 1 H, *J* = 10.1, 3.1 Hz, H-3′′), 3.74 (dd, 1 H, *J* =11.2, 7.3 Hz, H-6$_a$′′), 3.70 – 3.65 (m, 2 H, H-5′ and H-6$_b$′′), 3.58 – 3.50 (m, 4 H, H-4, H-3, H-3′ and H-2′), 3.46 (m, 1 H, H-5), 3.40 (ddd, 1 H, *J* = 8.5, 4.7, 3.6 Hz, H-2$_{Sp}$), 3.30 (m, 1 H, H-2), 2.10 (dm, 2 H, *J* = 126.9 Hz, H-6$_{Sp}$), 1.56 – 1.14 (m, 22 H, H-7$_{Sp}$ to H-17$_{Sp}$), 0.89 (t, 3 H, *J* = 7.0 Hz, H-18$_{Sp}$); ^{1}H NMR (400 MHz, $CDCl_3$, ^{13}C-decoupled) Chemical shifts are identical to the ^{1}H NMR presented for unlabeled globotriaosylsphingosine **26a**; ^{13}C NMR (151 MHz, MeOD-d_4) δ: 136.8 (d, *J* = 42.8 Hz, C-5$_{Sp}$), 128.3 (d, *J* = 72.3 Hz, C-4$_{Sp}$), 105.4 (C-1′), 103.7 (C-1), 102.7 (C-1′′), 80.8 (C-4), 79.8 (C-4′), 76.6 (C-5′ and C-5), 76.3 (C-2′), 74.65 (C-3), 74.2 (C-2), 72.8 (C-5′′), 72.6 (C-3′), 71.3 (C-4′′), 71.0 (C-3′′), 70.8 (d, *J* = 5.1 Hz, C-3$_{Sp}$), 70.5 (C-2′′), 67.1 (C-1$_{Sp}$), 62.7 (C-6′′), 61.6 (C-6), 61.5 (C-6′), 56.7 (d, *J* = 2.2 Hz, C-2$_{Sp}$), 33.8 – 32.9 (m, C-6$_{Sp}$ and $CH_{2\text{-}Sp}$), 30.9 – 29.8 (m, $CH_{2\text{-}Sp}$ x10), 23.7 ($CH_{2\text{-}Sp}$), 14.4 (C-18$_{Sp}$); HRMS Calcd for $[C_{31}{}^{13}C_5H_{67}NO_{17} + H]^+$: 791.4650, found 791.4654. **Nor-globotriaosyl-[5,6,7,8-$^{13}C_4$]-sphingosine (26*b)** HRMS Calcd for $[C_{31}{}^{13}C_4H_{67}NO_{17} + H]^+$: 776.4459, found 776.4463.



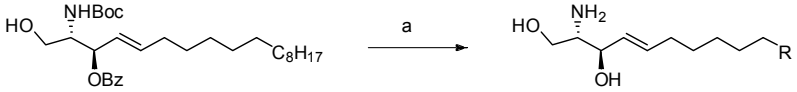
D-*erythro*-Sphingosine (27a-c) 3-*O*-benzoyl-*N*-(*tert*-butyloxycarbonyl)-D-*erythro*-sphingosine **(23)** (90 mg, 0.18 mmol, 1.0 eq) was dissolved in a methanol (6 mL) and sodium methoxide (30% in methanol) (12 µL, 0.09 mmol, 0.5 eq) was added. The reaction was stirred at room temperature until TLC showed full conversion to a lower running spot. Potassium hydroxide (0.5 M in water) (0.72 mL, 0.36 mmol, 2.0 eq) was added and the reaction was stirred over night at ambient temperature. The reaction was then quenched with acetic acid (0.05 mL, 0.89 mmol, 5.0 eq), before concentration *in vacuo*. The residue was co-evaporated once with toluene (10 mL) and then cooled to 0 °C before addition of TFA (2 mL). The reaction was stirred for 2 minutes at 0 °C and was then diluted with toluene (40 mL) and concentrated *in vacuo*. Purification by HPLC–MS (52–62 % B, following the general procedure for HPLC–MS purifications) produced the title compound (28 mg, 68 µmol, 38 %), its nor-homologue (11 mg, 28 µmol, 15 %) and di-nor-homologue (1 mg, 2.6 µmol, 1 %) as their respective TFA adducts. **(*E*)-(2*R*,3*S*)-2-Amino-octadec-4-ene-1,3-diol (27a)** $[\alpha]_D^{22}$: −2.0 (C = 0.5 MeOH); ^{1}H NMR (600 MHz, MeOD-d_4) δ: 5.85 (dtd, 1 H, *J* = 15.4, 6.9, 1.2 Hz, H-5), 5.47 (ddt, 1 H, *J* = 15.4, 6.9, 1.5 Hz, H-4), 4.28 (ddd, 1 H, *J* = 6.9, 4.7, 1.2 Hz, H-3), 3.79 (dd, 1 H, *J* = 11.6, 4.0 Hz, H-1$_a$), 3.66 (dd, 1 H, *J* = 11.6, 8.4 Hz, H-1$_b$), 3.20 (ddd, 1 H, *J* = 8.4, 4.7, 4.0 Hz, H-2), 2.10 (q, 2 H, *J* = 7.1 Hz, H-6), 1.45 – 1.39 (m, 2 H, H-7), 1.35 – 1.26 (m, 20 H, H-8 to H-17), 0.90 (t, 3 H, *J* = 7.0 Hz, H-18); ^{13}C NMR (151 MHz, MeOD-d_4) δ: 136.6 (C-5), 128.5 (C-4), 71.0 (C-3), 59.4 (C-1), 58.5 (C-2), 33.4 (C-6), 33.1, 30.81, 30.80 (2x CH_2), 30.77, 30.75, 30.65, 30.5, 30.4 (CH_2 x9), 30.2 (C-7), 23.8 (CH_2), 14.4 (C-18); IR (neat): 3289, 2918, 2850, 1668, 1520, 1470, 1192, 1134, 968, 720 cm^{-1}; HRMS Calcd for $[C_{18}H_{37}NO_2 + H]^+$: 300.2897, Found 300.2899. **(*E*)-(2*R*,3*S*)-2-Amino-heptadec-4-ene-1,3-diol (27b)** ^{1}H NMR (600 MHz, MeOD-d_4) δ: 5.85 (dtd, 1 H, *J* = 15.4, 6.9, 1.2 Hz, H-5), 5.47 (ddt, 1 H, *J* = 15.4, 6.9, 1.5 Hz, H-4), 4.28 (ddd, 1 H, *J* = 6.9, 4.7, 1.2 Hz, H-3), 3.79 (dd, 1 H, *J* = 11.6, 4.0 Hz, H-1$_a$), 3.66 (dd, 1 H, *J* = 11.6, 8.4 Hz, H-1$_b$), 3.20 (ddd, 1 H, *J* = 8.4, 4.7, 4.0 Hz, H-2), 2.10 (q, 2 H, *J* = 7.1 Hz, H-6), 1.45 – 1.39 (m, 2 H, H-7), 1.35 – 1.26 (m, 18 H, H-8 to H-16), 0.90 (t, 3 H, *J* = 7.0 Hz, H-17); ^{13}C NMR (151 MHz, MeOD-d_4) δ: 136.6 (C-5), 128.5 (C-4),

71.0 (C-3), 59.4 (C-1), 58.5 (C-2), 33.4 (C-6), 33.1, 30.81, 30.79, 30.76, 30.75, 30.64, 30.5, 30.4 (CH_2 x8), 30.2 (C-7), 23.8 (CH_2), 14.4 (C-17); IR (neat): 3289, 1918, 2850, 1668, 1525, 1436, 1185, 1136, 972, 725 cm^{-1}; HRMS Calcd for $[C_{17}H_{35}NO_2 + H]^+$: 286.2741, found 286.2742. **(*E*)-(2*R*,3*S*)-2-Amino-hexadec-4-ene-1,3-diol (27c)** ^1H NMR (600 MHz, MeOD-d_4) δ: 5.85 (dtd, 1 H, *J* = 15.4, 6.9, 1.2 Hz, H-5), 5.47 (ddt, 1 H, *J* = 15.4, 6.9, 1.5 Hz, H-4), 4.28 (dd, 1 H, *J* = 6.9, 4.7 Hz, H-3), 3.79 (dd, 1 H, *J* = 11.6, 4.0 Hz, H-1_a), 3.66 (dd, 1 H, *J* = 11.6, 8.4 Hz, H-1_b), 3.20 (ddd, 1 H, *J* = 8.4, 4.7, 4.0 Hz, H-2), 2.10 (q, 2 H, *J* = 7.1 Hz, H-6), 1.45 – 1.39 (m, 2 H, H-7), 1.35 – 1.26 (m, 16 H, H-8 to H-15), 0.90 (t, 3 H, *J* = 7.0 Hz, H-16); ^{13}C NMR (151 MHz, MeOD-d_4) δ: 136.6 (C-5), 128.5 (C-4), 71.0 (C-3), 59.4 (C-1), 58.5 (C-2), 33.4, 33.1, 30.81, 30.77, 30.75, 30.65, 30.5, 30.4, 30.2, 23.8 (CH_2 x10), 14.4 (C-16); HRMS Calcd for $[C_{16}H_{33}NO_2 + H]^+$: 272.2554, found 272.2586.

References and footnotes

1. Dakin, H. D. *J. Biol. Chem.* **1908,** *4* (6), 419-435.
2. Urey, H. C.; Brickwedde, F. G.; Murphy, G. M. *Physical Review* **1932,** *39* (1), 164.
3. Schoenheimer, R.; Rittenberg, D. *J. Biol. Chem.* **1935,** *111* (1), 163-168.
4. Schoenheimer, R.; Ratner, S.; Rittenberg, D. *J. Biol. Chem.* **1939,** *127* (1), 333-344.
5. Bequette, B. J.; Sunny, N. E.; El-Kadi, S. W.; Owens, S. L. *J. Anim Sci.* **2006,** *84* (13), 50-59.
6. Foster, M. P.; McElroy, C. A.; Amero, C. D. *Biochemistry* **2006,** *46* (2), 331-340.
7. Mackenzie, N.; Malthouse, J.; Scott, A. *Science* **1984,** *225* (4665), 883-889.
8. Dawadi, P. B. S.; Lugtenburg, J. *Molecules* **2010,** *15* (3), 1825-1872.
9. Kint, J. A. *Science* **1970,** *167* (3922), 1268-1269.
10. Aerts, J. M.; Groener, J. E.; Kuiper, S.; Donker-Koopman, W. E.; Strijland, A.; Ottenhoff, R.; van Roomen, C.; Mirzaian, M.; Wijburg, F. A.; Linthorst, G. E.; Vedder, A. C.; Rombach, S. M.; Cox-Brinkman, J.; Somerharju, P.; Boot, R. G.; Hollak, C. E.; Brady, R. O.; Poorthuis, B. J. *Proc. Natl. Acad. Sci. U. S. A.* **2008,** *105* (8), 2812-2817.
11. The labeled sphingolipid, can function as a precursor for both ceramide and glycosphingolipid synthesis.
12. Dagan, A.; Agmon, V.; Gatt, S.; Dinur, T.; Alfred H. Merrill, J. Y. A. H. *Methods Enzymol.* **2000,** *312*, 293-304.
13. Singh, R. D.; Puri, V.; Valiyaveettil, J. T.; Marks, D. L.; Bittman, R.; Pagano, R. E. *Mol. Biol. Cell* **2003,** *14* (8), 3254-3265.
14. Dueker, S. R.; Jones, A. D.; Smith, G. M.; Clifford, A. J. *Anal. Chem.* **1994,** *66* (23), 4177-4185.
15. Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998,** (8), 1075-1091.
16. Shapiro, D.; Segal, K. *J. Am. Chem. Soc.* **1954,** *76* (22), 5894-5895.
17. Kim, S.; Lee, S.; Lee, T.; Ko, H.; Kim, D. *J. Org. Chem.* **2006,** *71* (22), 8661-8664.
18. van den Berg, R. J. B. H. N.; Korevaar, C. G. N.; Overkleeft, H. S.; van der Marel, G. A.; van Boom, J. H. *J. Org. Chem.* **2004,** *69* (17), 5699-5704.
19. Schmidt, R. R.; Zimmermann, P. *Tetrahedron Lett.* **1986,** *27* (4), 481-484.
20. Li, Y. L.; Wu, Y. L. *Liebigs Annalen* **1996,** (12), 2079-2082.
21. Kiso, M.; Nakamura, A.; Tomita, Y.; Hasegawa, A. *Carbohydr. Res.* **1986,** *158*, 101-111.
22. Hirata, N.; Yamagiwa, Y.; Kamikawa, T. *J. Chem. Soc., Perkin Trans. 1* **1991,** (9), 2279-2280.
23. S Yadav, J.; Vidyanand, D.; Rajagopal, D. *Tetrahedron Lett.* **1993,** *34* (7), 1191-1194.
24. Somfai, P.; Olsson, R. *Tetrahedron* **1993,** *49* (30), 6645-6650.
25. Nicolaou, K. C.; Caulfield, T.; Kataoka, H.; Kumazawa, T. *J. Am. Chem. Soc.* **1988,** *110* (23), 7910-7912.
26. Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999,** *38* (11), 1532-1568.

27. Garner, P.; Park, J. M. *J. Org. Chem.* **1987,** *52* (12), 2361-2364.
28. Murakami, T.; Furusawa, K. *Tetrahedron* **2002,** *58* (45), 9257-9263.
29. Azuma, H.; Tamagaki, S.; Ogino, K. *J. Org. Chem.* **2000,** *65* (11), 3538-3541.
30. Chun, J.; Li, G.; Byun, H.-S.; Bittman, R. *Tetrahedron Lett.* **2002,** *43* (3), 375-377.
31. Blot, V.; Jacquemard, U.; Reissig, H. U.; Kleuser, B. *Synthesis* **2009,** (5), 759-766.
32. Grubbs, R. H. *Tetrahedron* **2004,** *60* (34), 7117-7140.
33. Campbell, A. D.; Raynham, T. M.; Taylor, R. J. K. *Synthesis* **1998,** (12), 1707-1709.
34. Coleman, R. S.; Carpenter, A. J. *Tetrahedron Lett.* **1992,** *33* (13), 1697-1700.
35. Seyferth, D.; Weiner, M. A. *J. Am. Chem. Soc.* **1961,** *83* (17), 3583-3586.
36. A reaction sequence without isolation of the intermediate Garner aldehyde gave an overall higher yield. Noteworthy is that the *syn*- and *anti*-isomers are troublesome to separate.
37. Cambridge Isotope Laboratories, Inc.: http://www.isotope.com (accessed Sept 12, 2010)
38. Gebhard, R.; Vanderhoef, K.; Lefeber, A. W. M.; Erkelens, C.; Lugtenburg, J. *Rec. Trav. Chim. Pays-Bas* **1990,** *109* (6), 378-387.
39. The procedure was slightly modified: The first equiv. of LDA from the literature procedure, was replaced with *n*-BuLi to avoid attack of the phosphochloridate by the formed diisopropylamine. This alteration gave a higher yield in this case as compared to when LDA was used.
40. Ouwerkerk, N.; van Boom, J. H.; Lugtenburg, J.; Raap, J. *Eur. J. Org. Chem.* **2000,** (5), 861-866.
41. Groesbeek, M.; Rood, G. A.; Lugtenburg, J. *Rec. Trav. Chim. Pays-Bas* **1992,** *111* (3), 149-154.
42. Oxalylchloride was chosen over oxalylbromide due to a beneficial difference in boilingpoints. bromoacetylchloride/oxalylchloride (127 °C/63 °C) bromoacetylbromide/oxalylbromide (150 °C/105 °C)
43. Bhattacharya, A. K.; Thyagarajan, G. *Chem. Rev.* **1981,** *81* (4), 415-430.
44. Olefin **20** can easily be acquired from tetradecanol via a two-step synthesis (Dess-Martin oxidation followed by Wittig methenylation).
45. Wu, D.; Xing, G. W.; Poles, M. A.; Horowitz, A.; Kinjo, Y.; Sullivan, B.; Bodmer-Narkevitch, V.; Plettenburg, O.; Kronenberg, M.; Tsuji, M.; Ho, D. D.; Wong, C. H. *Proc. Natl. Acad. Sci. U. S. A.* **2005,** *102* (5), 1351-1356.
46. Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980,** *19* (9), 731-732.
47. Evans, E. F.; Lewis, N. J.; Kapfer, I.; Macdonald, G.; Taylor, R. J. K. *Synth. Commun.* **1997,** *27* (11), 1819-1825.
48. This was performed to avoid possible benzoylation of the amine during the coevaporation with toluene in the following deprotection step.
49. Examination of the (nor)homologue mixtures by ^{1}H or ^{13}C NMR, cannot be used as an analytical tool for the quantification of the amount or identity of the (nor)homologues. The aliphatic region in both ^{1}H and ^{13}C NMR are not accurate enough for the exact integration or peak picking, especially in the case of ^{13}C NMR of the labeled compounds that have complex ^{13}C-^{13}C couplings in this region.
50. To conclude the existence of nor-homologues was sphingosine **23** deprotected. Purification by HPLC–MS produced D-*erythro*-sphingosine **27a** and its two nor-and di-nor-homologues **27b** and **27c** with identical product ratio as for lysoCTH **26a** and its nor- and di-nor-homologues **26b** and **26c**. The identity of sphingosine homologues **27a-c** was further confirmed by ^{1}H and ^{13}C NMR.



23

27a R = C_8H_{17}
27b R = C_7H_{15}
27c R = C_6H_{13}

51. Elimination of ^{13}C-atoms from the olefin is consistent with double bond isomerization prior to cross metathesis, eliminating propylene in place of ethylene.
52. The only visible difference is a slight change in integral for the lipophilic tail in proton NMR. Some of the carbon peaks in the aliphatic region of the^{13}C NMR spectra are not resolved.
53. Rai, A. N.; Basu, A. *Org. Lett.* **2004,** *6* (17), 2861-2863.
54. Torssell, S.; Somfai, P. *Org. Biomol. Chem.* **2004,** *2* (11), 1643-1646.
55. Nussbaumer, P.; Ettmayer, P.; Peters, C.; Rosenbeiger, D.; Hogenauer, K. *Chem. Commun.* **2005,** (40), 5086-5087.
56. Yamamoto, T.; Hasegawa, H.; Hakogi, T.; Katsumura, S. *Org. Lett.* **2006,** *8* (24), 5569-5572.
57. Peters, C.; Billich, A.; Ghobrial, M.; Hogenauer, K.; Ullrich, T.; Nussbaumer, P. *J. Org. Chem.* **2007,** *72* (5), 1842-1845.
58. Ghosal, P.; Shaw, A. K. *Tetrahedron Lett. 51* (31), 4140-4142.
59. Schmidt, B. *Eur. J. Org. Chem.* **2004,** *2004* (9), 1865-1880.
60. It is believed that products derived from thermal decomposition of ruthenium catalysts can facilitate double-bond isomerization. Hong *et al. J. Am. Chem. Soc.* **2004**, *126* (*24*), 7414. During the work described in this chapter, an article was published describing the formation of homologues during cross-metathesis of lipid-like structures during the synthesis of a fluorescent form of Vitamine E: West *et al. J. Org. Chem.* **2010**, *75* (*9*), 2883.
61. Chatterjee, A. K.; Choi, T.-L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003,** *125* (37), 11360-11370.
62. The commercial compound is extracted from porcine blood and analysis shows that it is of questionable purity.
63. Except for their difference in weight that is.
64. Ibuka, T.; Habashita, H.; Otaka, A.; Fujii, N.; Oguchi, Y.; Uyehara, T.; Yamamoto, Y. *J. Org. Chem.* **1991,** *56* (14), 4370-4382.
65. Herold, P. *Helv. Chim. Acta* **1988,** *71* (2), 354-362.
66. Lankalapalli, R. S.; Ouro, A.; Arana, L.; Goì mez-Munì*f*oz, A.; Bittman, R. *J. Org. Chem.* **2009,** *74* (22), 8844-8847.
67. Peppe, C.; de Azevedo Mello, P.; das Chagas, R. P. *J. Organomet. Chem.* **2006,** *691* (11), 2335-2339.
68. Siebum, A. H. G.; Woo, W. S.; Lugtenburg, J. *Eur. J. Org. Chem.* **2003,** (23), 4664-4678.

Chapter 4

Synthesis of Fluorogenic Hyaluronan Dimers as Hyaluronidase Probes

Introduction

Glycosaminoglycans (GAGs) are a family of glycans involved in many physiological and pathological processes.[1] GAGs are constructed from repeating disaccharide units, which consist of a 2-amino-2-deoxy glycoside and either a hexose moiety (as in keratan sulfate) or hexuronic acid moiety (as in chondroitin sulfate, dermatan sulfate, heparan sulfate/heparin and hyaluronan). GAGs are in most cases covalently bound to proteins, thereby forming proteoglycans, which are located in the extracellular matrix (ECM) of vertebrate cells. All GAGs are sulfated polymers, with the exception of hyaluronan. At extracellular pH (7.0-7.4) both the carboxylic acids and sulfates are deprotonated, giving the GAGs an anionic character. This property ensures for the attraction of water molecules (increasing the polymer volume of up to 1000 times) explaining the typical viscous form and the lubricating property of GAGs.

Hyaluronan is a unique member of the GAG-family from several points of view. It has the simplest primary structure of all the GAGs and is the only non-sulfated GAG species. It is additionally not covalently bound to any protein. Also, in contrast to the other GAGs is hyaluronan synthesized by integral membrane proteins at the cell surface and not as a posttranslational modification of proteins in the Golgi apparatus.[2] The biosynthesis of hyaluronan is performed by hyaluronan synthases, forming a linear polysaccharide, consisting of the [-*β*-1,4-D-glucuronic acid-*β*-1,3-*N*-acetyl-D-glucosamine-]$_n$ repeating unit, where n can reach 50,000 disaccharide units giving a molecular mass of up to 20 MDa.[3] Despite its relatively simple primary structure, the biological effects and activities of different length of hyaluronic acid fragments show

great diversity.[2] Macromolecular hyaluronan (MDa range) has many important functions, which includes being an immobilizer of water and radical scavenger in skin tissue. It is a lubricant and shock absorber in joints and functions as a visco-elastic component of the vitreous humour of the eye. The various effects of different size hyaluronan fragments may be best exemplified by their respective responses in tissue injury and repair processes.[4] For instance, high-molecular mass hyaluronan (>200 kDa) appears to have anti-angiogenic[5], immunosuppressive/anti-inflammatory[6] properties and has been shown to inhibit phagocytosis[7]. Interestingly, while high-molecular mass hyaluronan promotes tissue integrity and resilience, medium to low-molecular weight hyaluronan has the opposite effect. Medium-molecular mass hyaluronan (~200 kDa) induces inflammatory response by induction of chemokines[8] and low-molecular weight hyaluronan (~3 kDa) has a stimulating effect on angiogenesis[9]. Moreover, low-molecular hyaluronan fragments are also produced by malignant cells to facilitate tumor cell-invasion and motility and are thus associated with the progression of many cancers.[10] Hyaluronan also plays an important part in the pathology of several vascular pathologies, such as atherosclerosis.[11-13]

To understand these processes better, access to defined length hyaluronan fragments, as well as probes for the monitoring of the biosynthesis and biodegradation of hyaluronan are of importance. As mentioned in **Chapter 1** five different hyaluronidases exist that are responsible for the degradation of hyaluronan. Both Hyal1 and Hyal2 are widely distributed throughout the body and are the major contributors for the degradation of hyaluronan. Hyal2, a membrane bound protein mainly produces 20 kDa hyaluronan fragments (~ 100 saccharide residues), which are further degraded by Hyal1 located in the lysosome to predominantly tetrasaccharides.[14] The absence of easy accessible and efficient probes for the investigation of hyaluronidase activity is a drawback for this research. Synthesis of a hyaluronic acid construct, bearing an anomeric fluorophore may provide a tool that directly can measure hyaluronidase activity in fluorometric assays. Since hyaluronidases also exhibit transglycosidase activity, the removal of the non-reducing end C4-hydroxyl functionality of the glucuronic acid will prevent such an action. A presentation of the potential hyaluronidase probes is depicted in Figure 1.

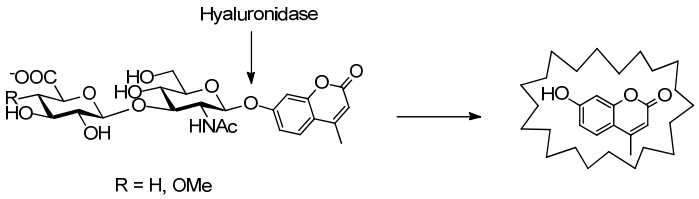


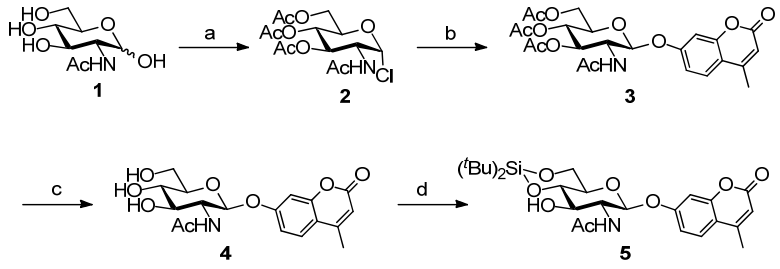
Figure 1. Fluorometric probe for the investigation of hyaluronidase activity.

This chapter describes the synthesis of three modified hyaluronic acid dimers bearing an anomeric 4-methylumbelliferone for fluorometric read-out, which are evaluated as substrates for the bovine hyaluronidase as well as the human Hyal2.

Results and Discussion

The synthesis of hyaluronic acid dimers **25**–**27** bearing a 4-methylumbelliferone (4MU) can be accessed by two possible routes. The 4MU can either be introduced at the dimer stage or at the monomer stage, followed by condensation with a suitable glucuronic acid donor. The modification at the C4-OH of the glucuronic acid makes the latter strategy the most efficient route, enabling access to the different target probes. Generally, either glycosyl halides or glycosyl imidates are applied as donors for aromatic *O*-glycosylation reactions.[15] GlcNAc-*α*-chloride **2** (Scheme 1) was therefore synthesised following the method of Horton *et al.*[16] Peracetylated GlcNAc-4MU **3** was then obtained by glycosylation of 4-methylumbelliferone with glycosyl chloride **2** under phase transfer conditions, using tetrabutylammonium bromide and cesium hydroxide. Reaction of glycosyl chloride **2** with the sodium salt of 4MU in either DMF[17] or under phase transfer conditions with 15-crown-5[18] were both less productive for the synthesis of GlcNAc-fluorophore **3**. Deprotection of the acetyl protecting groups of **3** using hydroxide reagents resulted in destruction of the fluorophore due to ring-opening of the lactone forming *cis*-coumarinic acid.[19] No ring-opening of the lactone was observed when the saponification of the acetyls was realized with sodium methoxide in methanol, giving access to the GlcNAc triol **4**. Protection of the 4,6-diol with di-*tert*-butylsilylene[20] produced the final GlcNAc-4MU acceptor **5** in 68% yield over three steps, starting from glycosyl chloride **2**. The di-*tert*-butylsilylene protective group[21] was chosen over the more commonly used 4,6-benzylidene, due to its stability and enhanced solubility, resulting in favorable acceptor properties.

Scheme 1. Synthesis of GlcNAc-4MU acceptor **5**.

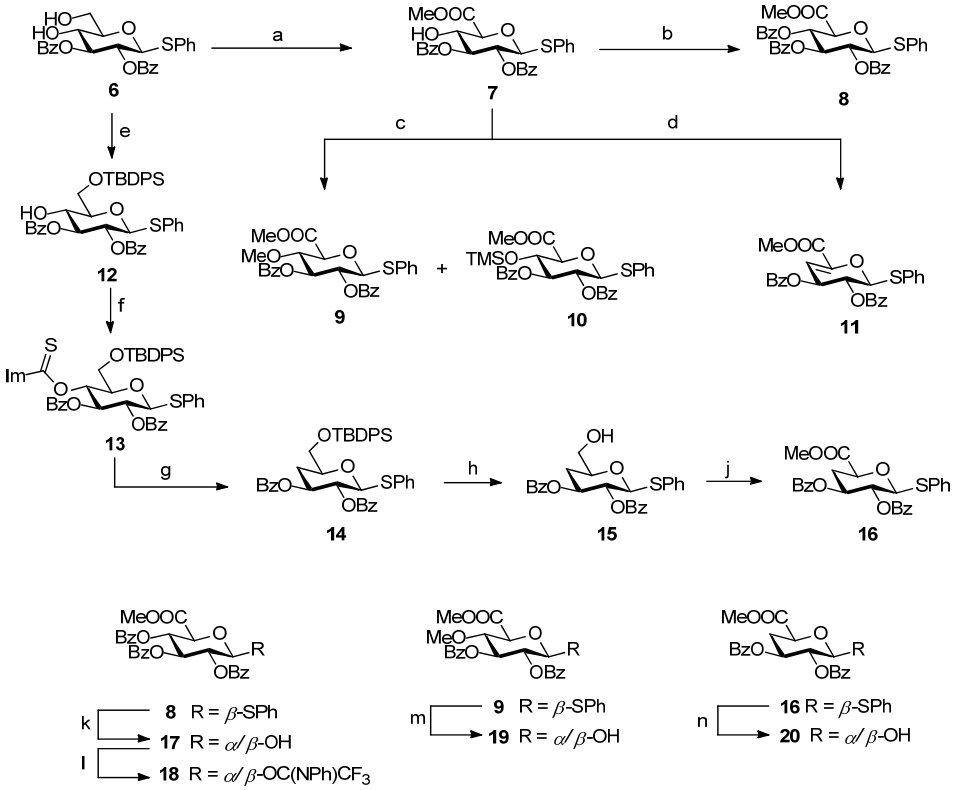


Reagents and conditions: [a] Horton *et al.*[16] [b] TBABr, CsOH, 4MU, DCM/water, r.t., 1 h, 72%. [c] NaOMe, MeOH, r.t., 4 h, 96%. [d] $(^{t}Bu)_2Si(OTf)_2$, pyridine, DMF, −40 °C, 45 min., 98%.

Phenyl thio-glycoside **6**[22] was employed as starting material for the synthesis of all three glucuronic acid building blocks (**8**, **9** and **16**, Scheme 2). Selective oxidation of the primary alcohol in diol **6** using TEMPO/BAIB[23] followed by methylation of the crude intermediate glucuronic acid with TMS-diazomethane in DCM/methanol afforded methyl ester **7**. Benzoylation of the C4-OH of **7** gave perbenzoylated thio-glucuronic acid donor **8**. Methylation of the same alcohol using various basic conditions resulted in partial C3-OBz to C4-OBz migration. For instance silver(I)oxide mediated methylation[24] gave a 4:1 mixture of C4-OMe *vs.* C3-OMe products. Contrary,

methylation of the C4-OH using acidic conditions (*i.e.*, TMS-diazomethane in combination with $BF_3{\cdot}Et_2O$ as catalyst) produced the desired product without any detectable benzoyl migration. Unfortunately, the yield in this step was moderate due to silylation of the C4-OH of the starting glucuronic acid, to give the C4-OTMS product **10**. This product could be isolated, unless the reaction was quenched with acetic acid prior to workup, in which case the starting glucuronic acid **7** was recovered. A mechanistic explanation for this type of side-reaction, when using TMS-CHN_2, has been investigated in the methylation of carboxylic acids.[25] The apparent 1:1 distribution between product **9** and C4-OTMS product **10**, indicates a mechanism in which $BF_3{\cdot}Et_2O$ initially catalyses the reaction of starting alcohol **7** with TMS-CHN_2, forming diazomethane and C4-OTMS product **10**. The *in situ* produced diazomethane is subsequently activated by $BF_3{\cdot}Et_2O$ for the methylation of **7**.[26]

Scheme 2. Synthesis of a set of glucuronic acid donors.



Reagents and conditions: [a] i) TEMPO, BAIB, DCM/water, r.t., 2.5 h; ii) $TMSCHN_2$, DCM/MeOH, r.t., 20 min., 73%. [b] BzCl, pyridine/DCM, r.t., 20 h, 98%. [c] $TMSCHN_2$, $BF_3{\cdot}Et_2O$, DCM, −40 °C to r.t., 2 h, 49%. [d] Im_2CS, toluene, 90 °C, 20 h, 43%. [e] TBDPSCl, imidazole, DMF, r.t., 20 h, 97%. [f] Im_2CS, toluene, 90 °C, 20 h, 89%. [g] AIBN, $^nBu_3SnH$, toluene, 90 °C, 6 h. 83%. [h] TBAF, THF, r.t., 2 h, 94%. [j] i) TEMPO, BAIB, DCM/water, r.t., 1 h; ii) $TMSCHN_2$, DCM/MeOH, r.t., 20 min., 90%, [k] NBS, acetone/water, 0 °C, 1 h, 98%. [l] $ClC(NPh)CF_3$, Cs_2CO_3, acetone, 0 °C, 2 h., 76%. [m] NIS, TFA, DCM, 0 °C, 2.5 h, 75%. [n] NIS, TFA, DCM, 0 °C, 2.5 h, 96%.

In the synthesis of 4-deoxy donor **16**, formation of a Barton-McCombie precursor from **7** was unsuccessful, due to formation of elimination product **11**. An alternative route to synthesize **16** was followed, starting with selective protection of diol **6** with TBDPS-chloride in DMF, producing **12** (Scheme 2). Barton-McCombie precursor **13** was formed by reaction of **12** with 1,1′-thiocarbonyldiimidazole in refluxing toluene. Radical deoxygenation of **13** by application of AIBN and tributyltin hydride as hydrogen source gave 4-deoxy glucoside **14**, which subsequently was desilylated using TBAF in THF. Oxidation of the primary alcohol **15** with TEMPO/BAIB reagent couple, followed by methylation of the intermediate carboxylic acid afforded the 4-deoxy thioglycoside donor **16**.

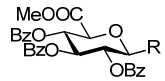
Glycosylation of *N*-acetyl glucosamine derivates with glucuronic acid donors have been reported with bromides[27,28] and phosphites[29] as anomeric leaving groups. The modest yields reported in these examples are most likely attributed to a relatively low reactivity of the glucuronic acid donors. To evaluate the best conditions for the condensation of GlcNAc acceptor **5** with different types of glucuronic acid donors, two additional donors were synthesized from thiodonor **8** (Scheme 2). Hydrolysis of **8** using NIS/TFA[30] produced hemiacetal **17** in a moderate yield, whereas application of NBS in wet acetone[31] resulted in a higher yield. Contrary, hydrolysis of 4-methoxy donor **9** and 4-deoxy donor **16** produced a higher yield using NIS/TFA hydrolysis as compared to NBS/wet acetone (Scheme 2). The hemiacetal **17** was then transformed into trifluoro *N*-phenylimidate donor **18**, using the standard procedure.[32]

The potent Tf_2O/Ph_2SO activation of anomeric thio-functionalities has successfully been utilized with a row of disarmed thiodonors[33] and was therefore evaluated with donor **8** and acceptor **5** (Table 1, entry 1). Although TLC analysis indicated complete activation of the donor at −40 °C no product could be isolated. The less reactive Tf_2O/BSP system[34] was then investigated with the same reaction partners (Table 1, entry 2). Full activation was achieved at −25 °C, producing an unidentified product spot that deteriorated over time. The same activation couple in presence of TTBP resulted in formation of orthoester **22** in 74% yield.[35] Next, a dehydrative glycosylation procedure[36] with 1-hydroxy donor **17** and GlcNAc-4MU acceptor **5** was investigated. Tf_2O/Ph_2SO activation of the anomeric hydroxyl functionality at −40 °C did form the desired product **21**, albeit in a modest yield. Increasing the activation temperature to either −20 °C or 0 °C showed an optimum for activation at −20 °C (Table 1, entries 4–6). Increasing the amount of donor to 2 equivalents (Table 1, entry 7) did not improve the yield any further. Application of the trifluoro *N*-phenylimidate donor **18** in combination with **5** with triflic acid as promoter resulted in the formation of **21** in 52% yield (Table 1, entry 8).[37]

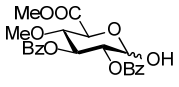
With the optimized condensation reaction in hand, hemiacetal donors **19** and **20** were utilized with the Ph_2SO/Tf_2O promoter system in a dehydrative coupling procedure forming hyaluronan disaccharides **23** and **24** (Table 1, entries 9 and 10) in 53% and 82% respectively.

Table 1. Coupling conditions for the synthesis of hyaluronan dimers **21**, **23** and **24**.

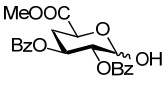
Donors



8 R = β-SPh
17 R = α/β-OH
18 R = α/β-OC(NPh)CF_3

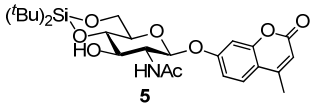


19



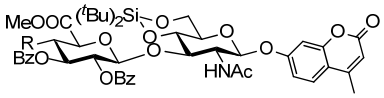
20

Acceptor

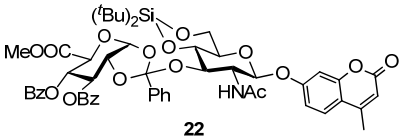


5

Products



21 R = OBz
23 R = OMe
24 R = H



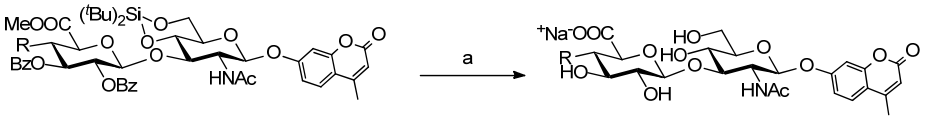
22

entry	donor[a]	acceptor	activator	Temp.	product	yield
1	**8**	**5**	Ph_2SO, Tf_2O	-40 °C	**21**	0%
2	**8**	**5**	BSP, Tf_2O	-25 °C	**21**	0%
3	**8**	**5**	BSP, Tf_2O, TTBP	-25 °C	**22**	74%
4	**17**	**5**	Ph_2SO, Tf_2O	-40 °C	**21**	18%
5	**17**	**5**	Ph_2SO, Tf_2O	-20 °C	**21**	74%
6	**17**	**5**	Ph_2SO, Tf_2O	-0 °C	**21**	30%
7	**17**	**5**	Ph_2SO, Tf_2O	-20 °C	**21**	53%
8	**18**	**5**	TfOH	0 °C	**21**	52%
9	**19**	**5**	Ph_2SO, Tf_2O	-20 °C	**23**	53%
10	**20**	**5**	Ph_2SO, Tf_2O	-20 °C	**24**	82%

[a] 1.2 equiv. for all entries but entry 7, for which 2 equiv. was used

Deprotection of the benzoyl esters in hyaluronan dimers **21**, **23** and **24** was accomplished with sodium methoxide in methanol/DCM, followed by removal of the di-*tert*-butylsilylene protecting group using hydrogen fluoride in pyridine. Saponification of the remaining methyl ester was performed under mild conditions using aqueous sodium carbonate (Scheme 3). Purification by RP–HPLC gave the three target hyaluronidase probes **25**, **26** and **27** in 73%, 63% and 77% yield respectively.[38]

Scheme 3. Deprotection of **21**, **23** and **24**, giving hyaluronidase probes **25**–**27**



21 R = OBz
23 R = OMe
24 R = H

25 R = OH
26 R = OMe
27 R = H

Reagents and conditions: [a] i) NaOMe cat., MeOH, DCM, o.n.; ii) HF·pyridine, pyridine, 6 h; iii) Na_2CO_3 aq., 4 h; iv) RP-HPLC, lyophilization and Amberlite-Na^+ **25**: 73%; **26**: 63%; **27**: 77%.

Hyaluronic acid-4MU probes **25** and **27** were tested for activity with hyaluronidase from bovine testes. Unfortunately, no hyaluronidase activity was detected with these probes. In a control experiment the compounds were tested with extracts from human spleen, containing the two glycosidases *exo*-*β*-glucuronidase and *exo*-*β*-*N*-acetyl-glucosaminidase. These enzymes degrade shorter hyaluronan fragments to the corresponding monosaccharides.[14] The *exo*-*β*-glucuronidase present in the spleen extract will first hydrolyze the GlcA-(*β*-1,3)-GlcNAc glycosidic linkage, before the *exo*-*β*-*N*-acetylglucosaminidase can cleave the glycosidic bond in GlcNAc-*β*-4MU (Figure 2). In this control experiment fluorescence was detected, especially with the C4′ non-modified probe **25**. This experiment shows that both **25** and **27** are viable substrates for the *exo*-glycosidases, but that the activity of the glucuronidase is somewhat hampered by the absence of the C4′-hydroxyl functionality.

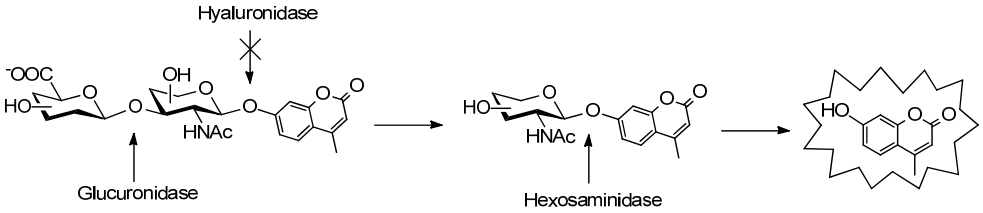


Figure 2. Enzymatic degradation of a hyaluronidase probe resulting in the release of 4MU.

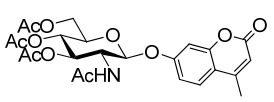
Conclusion

This chapter describes the synthesis of the potential hyaluronidase probes **25**–**27**. Key step in the synthetic route entailed the condensation between a disarmed glucuronic acid donor and a GlcNAc-4MU acceptor. Two of the probes were tested for hyaluronidase activity in a fluorometric assay. Although unaffected by the hyaluronidase enzyme, the probes proved to be sensitive to *exo*-glycosidase activity, when subjected to *exo*-*β*-glucuronidase and *exo*-*β*-*N*-acetylglucosaminidase indicating an insufficient affinity between the dimeric hyaluronic acid probe and the *endo*-hyaluronidase enzyme. The affinity for the hyaluronidase enzymes can be increased by elongating the hyaluronan chain, which will be the subject of **Chapter 5.**

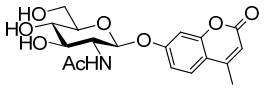
Experimental section

General Procedures and Material: Commercially available reagents and solvents (Acros, Fluka or Merck) were used as received unless stated otherwise. DCM and THF were freshly distilled before use over P_2O_5 and Na/benzophenone respectively. Triethylamine was destilled over calcium hydride and stored over KOH. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. All moisture sensitive reactions were performed under an atmosphere of argon. Traces of water was removed from starting compounds by coevaporation with DCE, dioxane and/or toluene. Molecular sieves 3Å were flamedried prior to use. Liquid column chromatography was performed using forced flow of the indicated solvent systems on Screening Devices Silica gel 60 (40–63 μm mesh). Size exclusion chromatography was performed on Sephadex LH20 (eluent MeOH/DCM, 1:1). Analytical TLC was performed on

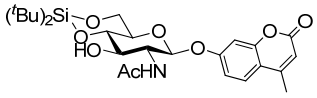
aluminium sheets, pre-coated with silica gel (Merck, silica gel 60, F_{254}). Compounds were visualized with UV absorption (245 nm), by spraying with either 20% H_2SO_4 in ethanol, or ammonium molybdate/cerium sulphate solution [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_6{\cdot}2H_2O$ (10 g/L), 10% sulphuric acid in ethanol], or phosphormolybdic acid in EtOH (150 g/L) followed by charring (~150 °C) or by spraying with an aqueous solution of potassium permanganate [$KMnO_4$ (20 g/L), K_2CO_3 (10 g/L)]. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter (Sodium D-line, λ = 589 nm). ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 (^1H) and 100.6 (^{13}C) MHz or on a Bruker AV 500 MHz spectrometer at 500.0 (^1H) and 125.1 (^{13}C) MHz respectively. Chemical shifts are reported as δ values (ppm) and directly referenced to TMS (0.00 ppm) in $CDCl_3$ or *via* the solvent residual peak (D_2O). Coupling constants (*J*) are given in Hz and all ^{13}C spectra are proton decoupled. NMR assignments were made using COSY and HSQC and in some cases TOCSY experiments. HPLC-MS analyses were performed on a LCQ Advantage Max (Thermo Finnigan) equipped with a Gemini C_{18} column (Phenomenex, 50 x 4.6 mm, 3μ), utilizing the following buffers: A: H_2O, B: acetonitrile and C: 1.0% $TFA_{(aq)}$. HPLC purifications were performed on a Gilson GX-281 automated HPLC system, equipped with a preparative Gemini C_{18} column (Phenomenex, 150 x 21.20, 5 μ). Products were eluted using the following buffers: A: ammonium acetate (20 $mM_{(aq)}$) or triethylammonium acetate (50 $mM_{(aq)}$), B: acetonitrile (HPLC-grade), 20 mL/min. Purified products were lyophilised on a CHRIST ALPHA 2–4 LD_{PLUS} to remove water and traces of buffer salts. Hyaluronidase enzyme was purchased from Sigma Aldrich (3506), Type I-S, originating from bovine testes, 290 units/mg solid. The assays were performed using Mac Ilvain buffer at pH 4.0, 5.0 and 6.0 and three different substrate concentrations: 0.1, 0.5 and 1.0 mM. 20–50 μg hyaluronidase or 25 μL water extract from spleen control. The assays were performed at 37 °C, during 2 hours and was then quenched by adding 0.3 M glycine-NaOH (pH 10.6). The fluorescence were measured at 366 nm (exc.) and 445 nm (em.).



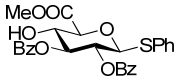
4-Methylumbelliferyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-*β*-D-glucopyranoside (3) 4-methylumbelliferone (2.2 g, 13 mmol, 1.5 eq) and tetrabutylammonium bromide (4.1 g, 13 mmol, 1.5 eq) were dissolved in DCM (30 mL). To this solution was cesium hydroxide (3.6 g, 12 mmol, 1.4 eq), dissolved in water (30 mL) added and the two-phase system was stirred vigorously for 10 minutes at room temperature. 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-*β*-D-glucopyranosyl chloride[16] (3.1 g, 8.5 mmol, 1.0 eq), dissolved in DCM (10 mL) was then added dropwise over 10 minutes. The mixture was stirred for an additional 45 minutes and was then transferred to an extraction funnel using DCM (20 mL) and washed with water (2x 100 mL) and brine (80 mL). The aqueous layers were extracted with DCM (100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (5–20% acetone in DCM) produced the title compound as a white solid (3.08 g, 6.09 mmol, 72%). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.56 (m, 1 H, H-$5_{4\text{-MU}}$), 7.01 – 6.96 (m, 2 H, H-$6_{4\text{-MU}}$ and H-$8_{4\text{-MU}}$), 6.18 (d, 1 H, *J* = 1.1 Hz, H-$3_{4\text{-MU}}$), 5.44 – 5.38 (m, 2 H, H-1 and H-3), 5.13 (dd, 1 H, *J* = 10.2, 9.4 Hz, H-2), 4.30 (dd, 1 H, *J* = 12.3, 5.7 Hz, H-6_a), 4.22 (dd, 1 H, *J* = 10.2, 8.3 Hz, H-4), 4.19 (dd, 1 H, *J* = 12.3, 2.3 Hz, H-6_b), 4.02 (ddd, 1 H, *J* = 10.2, 5.7, 2.3 Hz, H-5), 3.95 (s, 1 H, NH), 2.43 (s, 3 H, $CH_{3\text{-}4\text{-MU}}$), 2.13 (s, 3 H, $CH_{3\text{-OAc}}$), 2.09 (s, 6 H, $CH_{3\text{-OAc}}$ x2), 1.93 (s, 3 H, $CH_{3\text{-NAc}}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.5 ($C{=}O_{NAc}$), 170.8, 170.6, 169.6 ($C{=}O_{OAc}$ x3), 161.4, 159.42, 154.35, 153.0 (C-$2_{4\text{-MU}}$, C-$7_{4\text{-MU}}$, C-$9_{4\text{-MU}}$ and C-$10_{4\text{-MU}}$), 125.5 (C-$5_{4\text{-MU}}$), 114.9 (C-$4_{4\text{-MU}}$), 114.0 (C-$6_{4\text{-MU}}$), 112.2 (C-$3_{4\text{-MU}}$), 103.5 (C-$8_{4\text{-MU}}$), 97.7 (C-1), 72.0 (C-3), 71.8 (C-5), 68.4 (C-2), 61.9 (C-6), 53.8 (C-4), 22.3 ($CH_{3\text{-NAc}}$), 20.3, 20.17, 20.16 ($CH_{3\text{-OAc}}$ x3), 18.3 ($CH_{3\text{-}4\text{-MU}}$); IR (neat): 2959, 2932, 2860, 1718, 1616, 1267, 1072, 1034, 827, 766 cm^{-1}; HRMS Calcd for $[C_{24}H_{27}NO_{11} + Na]^+$: 528.1476, found 528.1474.



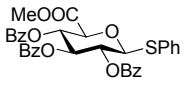
4-Methylumbelliferyl 2-acetamido-2-deoxy-*β*-D-glucopyranoside (4) Peracetylated *N*-acetyl glucosamine **3** (4.0 g, 7.9 mmol, 1.0 eq) was dissolved in MeOH: DCM 1:1 (250 mL) and sodium methoxide (30% in MeOH) (0.33 mL, 2.4 mmol, 0.3 eq) was added and reaction was left stirring at room temperature for 4 hours. The reaction was then quenched with Amberlite-H^+, filtered and concentrated *in vacuo*. The product was recovered without further purification in >95% purity according to ^{1}H-NMR (2.9 g, 7.6 mmol, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.65 (d, 1 H, *J* = 8.7 Hz, H-5$_{4\text{-MU}}$), 7.05 – 6.98 (m, 2 H, H-6$_{4\text{-MU}}$ and H-8$_{4\text{-MU}}$), 6.20 (d, 1 H, *J* = 1.0 Hz, H-3$_{4\text{-MU}}$), 5.18 (d, 1 H, *J* = 8.4 Hz, H-1), 4.00 – 3.91 (m, 2 H, H-2 and H-3), 3.76 (dd, 1 H, *J* = 12.0, 5.0 Hz, H-6$_a$), 3.61 (dd, 1 H, *J* = 10.4, 8.3 Hz, H-4), 3.53 – 3.45 (m, 2 H, H-5 and H-6$_b$), 2.46 (d, 3 H, *J* = 1.0 Hz, $CH_{3\text{-4-MU}}$), 2.00 (s, 3 H, $CH_{3\text{-NAc}}$); IR (neat): 3298, 2924, 2890, 1717, 1616, 1539, 1290, 1081, 1042, 853, 628 cm^{-1}; HRMS Calcd for $[C_{18}H_{21}NO_8 + H]^+$: 380.1340, found 380.1341.



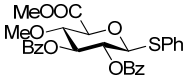
4-Methylumbelliferyl 2-acetamido-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-*β*-D-glucopyranoside (5) Glycoside **4** (2.8 g, 7.4 mmol, 1.05 eq) was coevaporated with anhydrous DMF and was then dissolved in anhydrous DMF (140 mL). The reaction was cooled to −40 °C, before dropwise addition of di-*tert*-butylsilanediyl bistriflate (2.3 mL, 7.0 mmol, 1.0 eq). The reaction was stirred for 30 minutes at −40 °C followed by addition of pyridine (1.7 mL, 21 mmol, 3.0 eq). The reaction was stirred an additional 15 minutes and was then transferred to an extraction funnel using diethyether (400 mL). The organics were washed with water (2x 400 mL) and brine (350 mL) The aqueous layers were extracted with ether (400 mL) and the combined organics were dried (Na_2SO_4), filtrated and concentrated *in vacuo*. The residue was purified with column chromatography (80–100% EtOAc in petroleum ether), giving the title compound as a white solid (3.58 g, 6.89 mmol, 98%). R_f = 0.41 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (d, 1 H, *J* = 8.8 Hz, H-5$_{4\text{-MU}}$), 7.23 (d, 1 H, *J* = 7.6 Hz, NH), 6.91 (dd, 1 H, *J* = 8.8, 2.0 Hz, H-6$_{4\text{-MU}}$), 6.87 (d, 1 H, *J* = 2.0 Hz, H-8$_{4\text{-MU}}$), 6.04 (s, 1 H, H-3$_{4\text{-MU}}$), 5.48 (d, 1 H, *J* = 7.9 Hz, H-1), 4.20 (dd, 1 H, *J* = 10.1, 4.9 Hz, H-6$_a$), 4.06 (s, 1 H, 3-OH), 4.04 – 3.98 (m, 3 H, H-2, H-3, H-6$_b$), 3.85 (dd, 1 H, *J* = 9.5, 8.1 Hz, H-4), 3.60 (ddd, 1 H, *J* = 9.7, 9.5, 5.3 Hz, H-5), 2.33 (s, 3 H, $CH_{3\text{-4-MU}}$), 2.09 (s, 3 H, $CH_{3\text{-NAc}}$), 1.06 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 0.99 (s, 9 H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.8 ($C{=}O_{NAc}$), 161.1, 159.7, 154.4, 152.6 (C-2$_{4\text{-MU}}$, C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 125.4 (C-5$_{4\text{-MU}}$), 114.6 (C-4$_{4\text{-MU}}$), 114.0 (C-6$_{4\text{-MU}}$), 112.2 (C-3$_{4\text{-MU}}$), 103.5 (C-8$_{4\text{-MU}}$), 98.1 (C-1), 77.2 (C-4), 74.3 (C-3), 70.5 (C-5), 66.0 (C-6), 56.3(C-2), 27.3, 26.8 ($CH_{3\text{-tBu-Si}}$ x2), 23.3 ($C_{q\text{-tBu-Si}}$), 22.5 ($CH_{3\text{-NAc}}$), 19.8 ($CH_{3\text{-4-MU}}$), 18.4 ($C_{q\text{-tBu-Si}}$); IR (neat): 3308, 2936, 2861, 1718, 1616, 1389, 1268, 1071, 827, 653 cm^{-1}; HRMS Calcd for $[C_{26}H_{37}NO_8Si + H]^+$: 520.2361, found 520.2363.



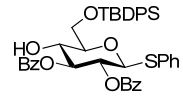
Methyl (phenyl 2,3-di-*O*-benzoyl-1-thio-*β*-D-glucopyranoside) uronate (7) A solution of diol **6** (1.6 g, 3.4 mmol, 1.0 eq), TEMPO (0,10 g, 0.66 mmol, 0.2 eq) and BAIB (2.6 g, 8.2 mmol, 2.5 eq) in DCM/water 2:1 (45 mL) was stirred vigorously for 2.5 hours and then quenched with $Na_2S_2O_3$ (10% aq.) (100 mL). The reaction was extracted with EtOAc (50 mL) and washed with brine (50 mL). The organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. The crude mixture was coevaporated with toluene two times, dissolved in DCM:MeOH 2:1 (50 mL). TMS-diazomethane (2 M in diethyl ether) (4.9 mL, 9.8 mmol, 3.0 eq) was added. The reaction was quenched with AcOH (2 mL), diluted with EtOAc (50 mL), washed with sat. aq. $NaHCO_3$ (2x 100 mL) and brine (100 mL). The organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (20–40% EtOAc in petroleum ether) produced the title compound as a white solid (1.3 g, 2.5 mmol, 73% over two steps). R_f = 0.13 (20% EtOAc in petroleum ether); NMR data are in accordance with literature precedence.[23] IR (neat): 2463, 3058, 2959, 1718, 1451, 1258, 1216, 1064, 708 cm^{-1}; HRMS Calcd for $[C_{27}H_{24}O_8S + Na]^+$: 531.1084, found 531.1081.



Methyl (phenyl 2,3,4-tri-*O*-benzoyl-1-thio-*β*-D-glucopyranoside) uronate (8) Methyl glucuronate **7** (0.82 g, 1.6 mmol, 1.0 eq) was dissolved in DCM/pyridine 1:1 (30 mL) and benzoyl chloride (0.37 mL, 4.0 mmol, 2.5 eq) was added to the solution at room temperature. The reaction was stirred for 4 hours and was then concentrated *in vacuo*. The residue was dissolved in EtOAc (100 mL) and washed with 1 M HCl (100 mL), sat. aq. $NaHCO_3$ (100 mL) and brine (100 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (40–100% DCM in petroleum ether) produced the title compound as a white solid (0.97 g, 1.6 mmol, 98%). R_f = 0.17 (60% DCM in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 – 7.94 (m, 2 H, H_{arom}), 7.93 – 7.90 (m, 2 H, H_{arom}), 7.83 – 7.80 (m, 2 H, H_{arom}), 7.55 – 7.48 (m, 4 H, H_{arom}), 7.45 – 7.31 (m, 8 H, H_{arom}), 7.29 – 7.25 (m, 2 H, H_{arom}), 5.92 (t, 1 H, *J* = 9.4 Hz, H-3), 5.64 (t, 1 H, *J* = 9.4 Hz, H-4), 5.49 (t, 1 H, *J* = 9.9 Hz, H-2), 5.49 (2, 1 H, *J* = 10.2 Hz, H-1), 4.36 (d, 1 H, *J* = 9.9 Hz, H-5), 3.71 (s, 3 H, $CH_{3\text{-}COOMe}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.9 ($C{=}O_{COOMe}$), 165.6, 165.1, 164.9 ($C{=}O_{Bz}$ x3), 133.5, 133.43, 133.35, 133.3 (CH_{arom} x4), 131.3 ($C_{q\text{-}arom}$), 129.84, 129.78 (CH_{arom} x2), 129.1 ($C_{q\text{-}arom}$), 129.0 (CH_{arom}), 128.7 ($C_{q\text{-}arom}$), 128.6, 128.4, 128.3 (CH_{arom} x3), 86.5 (C-1), 76.5 (C-5), 73.4 (C-3), 70.1 (C-2 and C-4), 52.9 ($CH_{3\text{-}COOMe}$); IR (neat): 3069, 2953, 2924, 1772, 1734, 1706, 1452, 1262, 1099, 1065, 1025, 700cm^{-1}; HRMS Calcd for $[C_{34}H_{28}O_9S + Na]^+$: 635.1346, found 635.1341.

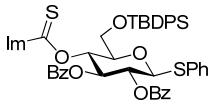


Methyl (phenyl 2,3-di-*O*-benzoyl-4-*O*-methyl-1-thio-*β*-D-glucopyranoside) uronate (9) To a solution of Methyl (phenyl glucopyranoside)uronate **7** (0.51 g, 1.0 mmol, 1.0 eq) in anhydrous DCM (5 mL), $BF_3{\cdot}OEt_2$ (0.25 mL, 2.0 mmol, 2.0 eq) and trimethylsilyldiazomethane (2 M in diethyl ether) (1.0 mL, 2.0 mmol, 2.0 eq) were added at −40 °C going to ambient temperature over two hours. The reaction was quenched with AcOH (0.5 mL), diluted with DCM (20 mL) and washed with sat. aq. $NaHCO_3$ (2x 50 mL) and brine (50 mL). The organics were dried ($MgSO_4$), filtrated and concentrated *in vacuo*. Purification by column chromatography (20% EtOAc in petroleum ether) afforded the title compound **9** as a white solid (0.25 g, 0.49 mmol, 49%, 92% based on recovered starting material). R_f = 0.45 (30% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.99 – 7.91 (m, 4 H, H_{arom}), 7.54 – 7.49 (m, 5 H, H_{arom}), 7.42 – 7.31 (m, 6 H, H_{arom}), 5.68 (t, 1 H, *J* = 9.2 Hz, H-3), 5.38 (t, 1 H, *J* = 9.6 Hz, H-2), 4.98 (d, 1 H, *J* = 9.6 Hz, H-1), 4.10 (d, 1 H, *J* = 9.6 Hz, H-5), 3.92 (t, 1 H, *J* = 9.2 Hz, H-4), 3.89 (s, 3 H, $CH_{3\text{-}COOMe}$), 3.42 (s, 3 H, $CH_{3\text{-}OMe}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.0 ($C{=}O_{COOMe}$), 165.5 ($C{=}O_{Bz}$), 165.1 ($C{=}O_{Bz}$), 133.3, 133.2 (CH_{arom} x2), 131.8 (C $C_{q\text{-}arom}$), 130.0, 129.8, 129.7 (CH_{arom} x3), 129.1 ($C_{q\text{-}arom}$), 128.9, 128.3 (CH_{arom} x2), 86.8 (C-1), 78.9 (C-4), 77.8 (C-5), 75.5 (C-3), 70.2 (C-2), 52.7 ($CH_{3\text{-}COOMe}$), 60.4 ($CH_{3\text{-}OMe}$); IR (neat): 3070, 2951, 1747, 1733, 1718, 1452, 1264, 1065, 1022, 709, 686 cm^{-1}; HRMS Calcd for $[C_{28}H_{26}O_8S + Na]^+$: 545.1241, found 545.1236.

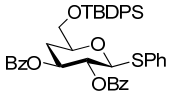


Phenyl 2,3-di-*O*-benzoyl-6-(*tert*-butyldiphenylsilyl)-1-thio-*β*-D-glucopyranoside (12) To a solution of phenyl thioglucoside diol **6**[22] (5.2 g, 11 mmol, 1.0 eq) in DMF (20 mL), was imidazole (1.5 g, 22 mmol, 2.0 eq) added followed by TBDPS-Cl (3.6 mL, 14 mmol, 1.3 eq). The reaction was quenched after 20 hours with MeOH (5 mL) and was then diluted with water (500 mL) and extracted with ether (2x 400 mL). The organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (5–25% EtOAc in petroleum ether) yielded the title compound as a colourless oil (7.55 g, 10.5 mmol, 97%). R_f = 0.63 (30% acetone in petroleum ether); $[\alpha]_D^{22}$: +56 (C = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 – 7.99 (m, 4 H, H_{arom}), 7.80 – 7.75 (m, 4 H, H_{arom}), 7.54 – 7.38 (m, 15 H, H_{arom}), 7.31 – 7.25 (m, 4 H, H_{arom}), 5.52 (t, 1 H, *J* = 9.2 Hz, H-3), 5.45 (t, 1 H, *J* = 9.2 Hz, H-2), 4.97 (d, 1 H, *J* = 9.6 Hz, H-1), 4.04 – 4.10 (m, 3 H, H-4, H-6_a, H-6_b), 3.71 (m, 1 H, H-5), 3.17 (bs, 1 H, OH), 1.13 (s, 9 H, $CH_{3\text{-}tBu}$),

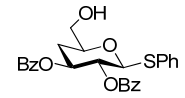
^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.4, 165.4 ($C{=}O_{Bz}$ x2), 135.8 (x2), 133.6, 133.4 (CH_{arom} x4), 132.9 ($C_{q\text{-}arom}$), 132.7(CH_{arom}), 132.6 ($C_{q\text{-}arom}$), 130.1, 130.00, 129.95 (CH_{arom} x3), 129.5, 129.1 ($C_{q\text{-}arom}$ x2), 129.0, 128.5, 128.1, 128.0 (CH_{arom} x4), 86.2 (C-1), 80.1 (C-5), 78.2 (C-3), 70.5, 70.2 (C-2, C-4), 64.2 (C-6), 27.0 ($CH_{3\text{-}tBu}$), 19.4 ($C_{q\text{-}tBu}$); IR (neat): 3494, 2930, 2858, 1728, 1067, 1275 734, 701, 502 cm^{-1}; HRMS Calcd for $[C_{42}H_{42}O_7SSi + Na]^+$: 741.2313, found 741.2313.



Phenyl 2,3-di-*O*-benzoyl-6-(*tert*-butyldiphenylsilyl)-4-thiocarbonylimidazole-1-thio-*β*-D-glucopyranoside (13) Phenyl thioglycoside **12** (6.7 g, 9.3 mmol, 1.0 eq) and Im_2CS (2.5 g, 14 mmol, 1.5 eq) were dissolved in anhydrous toluene (120 mL). The mixture was heated for 5 hours at 90 °C and cooled to room temperature. The reaction was washed with sat. aq. $NaHCO_3$ (200 mL) and brine (200 mL). The organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (30–50% EtOAc in petroleum ether) yielded the title product as a off-white solid (6.9 g, 8.3 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.16 (s, 1 H, H-$2_{imidazole}$), 7.95 (d, 2 H, *J* = 8.1 Hz, H_{arom}), 7.80 (d, 2 H, *J* = 8.1 Hz, H_{arom}), 7.70 (dd, 2 H, *J* = 8.0, 1.3 Hz, H_{arom}), 7.62 (dd, 1 H, *J* = 8.0, 1.3 Hz, H_{arom}), 7.55 – 7.48 (m, 3 H, *J* = 6.8 Hz, H-$5_{imidazole}$ and H_{arom} x2), 7.46 – 7.22 (m, 17 H, H_{arom}), 6.97 (dd, 1 H, *J* =1.6, 0.7 Hz, H-$4_{imidazole}$), 6.21 (t, 1 H, *J* = 9.6 Hz, H-4), 5.96 (t, 1 H, *J* = 9.5 Hz, H-3), 5.54 (t, 1 H, *J* = 9.8 Hz, H-2), 5.09 (d, 1 H, *J* = 10.0 Hz, H-1), 3.95 (m, 1 H, H-5), 3.91 (dd, 1 H, *J* = 11.8, 2.4 Hz, H-6_a) 3.85 (dd, 1 H *J* = 11.8, 4.7 Hz, H-6_b), 1.07 (s, 9 H, $CH_{3\text{-}tBu}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 182.4 (C=S), 165.7, 164.9 ($C{=}O_{Bz}$ x2), 137.0, 135.6, 135.4, 133.42, 133.35, 132.7 (CH_{arom} x6), 132.5, 132.2 ($C_{q\text{-}arom}$ x2), 130.9 ($CH_{imidazole}$), 129.82, 129.78, 129.0, 128.4, 128.3, 128.2, 127.7 (CH_{arom} x7), 118.0 ($CH_{imidazole}$), 86.5 (C-1), 78.9 (C-5), 76.5 (C-4), 74.2 (C-3), 70.2 (C-2), 62.7 (C-6), 26.7 ($CH_{3\text{-}tBu}$), 19.1 ($C_{q\text{-}tBu}$); IR (neat): 3070, 2934, 2860, 1734, 1393, 1270, 1221, 1068, 1026, 985, 703 cm^{-1}; HRMS Calcd for $[C_{46}H_{44}N_2O_7S_2Si + H]^+$: 829.2432, found 829.2439.

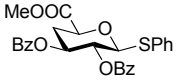


Phenyl 2,3-di-*O*-benzoyl-6-(*tert*-butyldiphenylsilyl)-4-deoxy-1-thio-*β*-D-glucopyranoside (14) Barton-McCombie precursor **13** (6.9 g, 8.3 mmol, 1.0 eq) was coevaporated with anhydrous toluene two times and was then dissolved in anhydrous toluene (100 mL). Bu_3SnH (5.5 mL, 21 mmol, 2.5 eq) and AIBN (0.20 g, 1.2 mmol, 0.15 eq) were added at 90 °C. The reaction was stirred at this temperature for two hours and was then cooled down before being washed with sat. aq. $NaHCO_3$ (100 mL) and brine (100 mL). The organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (30% EtOAc in petroleum ether) gave 4-deoxy glucose derivate **14** as an colorless oil (4.9 g, 6.9 mmol, 83%). $[\alpha]_D^{22}$: +60 (C = 1.0 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, 2 H, *J* = 7.8 Hz, H_{arom}), 7.94 (d, 2 H, *J* = 7.8 Hz, H_{arom}), 7.74 – 7.67 (m, 4 H, H_{arom}), 7.51 – 7.45 (m, 4 H, H_{arom}), 7.43 – 7.32 (m, 10 H, H_{arom}), 7.25 – 7.20 (m, 3 H, H_{arom}), 5.43 – 5.35 (m, 2 H, H-2, H-3), 4.94 (d, 1 H, *J* = 9.4 Hz, H-1), 3.89 – 3.83 (m, 2 H, H-5, H-6_a), 3.76 (m, 1 H, H-6_b), 2.44 (dd, 1 H, *J* = 12.4, 3.4 Hz, H-4_{eq}), 1.86 (dd, 1 H, *J* = 12.4, 11.5 Hz, H-4_{ax}), 1.14 (s, 9 H, $CH_{3\text{-}tBu}$), ^{13}C NMR (126 MHz, $CDCl_3$) δ: 165.8, 165.4 ($C{=}O_{Bz}$ x2), 135.60, 135.58, 133.12, 133.10 (CH_{arom} x4), 133.01 ($C_{q\text{-}arom}$), 132.3, 129.74, 129.68 (CH_{arom} x3), 129.6, 129.4 ($C_{q\text{-}arom}$ x2), 128.8, 128.3, 127.7, 127.5 (CH_{arom} x4), 86.4 (C-1), 76.6 (C-5), 73.2 (C-3), 71.2 (C-2), 66.0 (C-6), 32.9 (C-4), 26.8 ($CH_{3\text{-}tBu}$), 19.2 ($C_{q\text{-}tBu}$); IR (neat): 2931, 2857, 1718, 1451, 1428, 1274, 1070, 1027, 908, 733, 702, cm^{-1}; HRMS Calcd for $[C_{42}H_{42}O_6SSi + Na]^+$: 725.2364, found 725.2364.

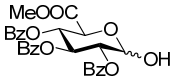


Phenyl 2,3-di-*O*-benzoyl-4-deoxy-1-thio-*β*-D-glucopyranoside (15) To a solution of 4-deoxy phenyl thioglycoside **14** (3.7 g, 5.2 mmol, 1.0 eq) in THF (40 mL) was TBAF (1 M in THF) (10.4 mL, 10.4 mmol, 2.0 eq) added at room temperature. The reaction was stirred for two hours and was then dissolved in EtOAc (200mL) and washed with sat. aq. $NaHCO_3$ (250 mL) and brine (200 mL). The aqueous layers

were extracted with EtOAc (200 mL) and the combined organics were dried (Na_2SO_4), filtrated and concentrated *in vacuo*. Purification by column chromatography (20–50% EtOAc in petroleum ether) produced the title compound as a white solid (2.28 g, 4.91 mmol, 94%). $[\alpha]_D^{22}$: +101 (C = 1.0 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, 2 H, *J* = 7.4 Hz, H_{arom}), 7.92 (d, 2 H, *J* = 7.4 Hz, H_{arom}), 7.55 – 7.45 (m, 4 H, H_{arom}), 7.41 – 7.33 (m, 4 H, H_{arom}), 7.32 – 7.28 (m, 3 H, H_{arom}), 5.44 – 5.36 (m, 2 H, H-2, H-3), 4.96 (d, 1 H, *J* = 10.0 Hz, H-1), 3.85 (m, 1 H, H-5), 3.76 (ddd, 1 H, *J* = 11.4, 7.8, 3.2 Hz, H-6_a), 3.68 (dt, 1 H, *J* = 11.4, 5.9 Hz, H-6_a), 2.33 (m, 1 H, H-4_{eq}), 2.16 (dd, 1 H, *J* = 7.8, 5.9 Hz, OH), 1.82 (m, 1 H, H-4_{ax}); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 165.8, 165.4 ($C{=}O_{Bz}$ x2), 132.6, 133.4 (CH_{arom} x2), 132.3 ($C_{q\text{-}aorm}$), 129.8, 129.7 (CH_{arom} x2), 129.5, 129.4 ($C_{q\text{-}aorm}$ x2), 128.2, 128.5, 129.1 (CH_{arom}x3), 86.3 (C-1), 76.6 (C-5), 73.0 (C-3), 71.2 (C-2), 65.0 (C-6), 32.3 (C-4); IR (neat): 3513, 2931, 2872, 1718, 1451, 1274, 1068, 1026, 908, 748, 705 cm^{-1}; HRMS Calcd for $[C_{26}H_{24}O_6S + Na]^+$: 487.1186, found 487.1183.

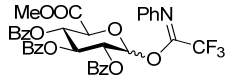


Methyl (phenyl 2,3-di-*O*-benzoyl-4-deoxy-1-thio-*β*-D-glucopyranoside) uronate (16) A solution of phenyl thioglucoside **15** (2.0 g, 4.4 mmol, 1.0 eq), TEMPO (0.14 g, 0.87 mmol, 0.2 eq) and BAIB (3.5 g, 11 mmol, 2.5 eq), in DCM:H_2O 2:1 (45 mL) was stirred vigorously for one hour. The reaction was then quenched with $Na_2S_2O_3$ (10% aq.) (150 mL) and the mixture was extracted with EtOAc (2x 150 mL). The organics were then washed with water (150 mL) and brine (150 mL), before being dried ($MgSO_4$), filtrated and concentrated *in vacuo*. The crude mixture was coevaporated with toluene two times and dissolved in DCM:MeOH 2:1 (45 mL). TMS-diazomethane (2 M in diethyl ether) (6.5 mL, 13 mmol, 3.0 eq) was added. The reaction was then quenched with AcOH (5 mL), diluted with EtOAc (100 mL), washed with sat. aq. $NaHCO_3$ (2x 100 mL) and brine (100 mL). The aqueous layers were extracted with EtOAc (100mL) and the combined organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (20–40% EtOAc in petroleum ether) yielded the title methyl uronate as a white solid (1.9 g, 4.1 mmol, 90% over two steps). $[\alpha]_D^{22}$: +78 (C = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, 2 H, *J* = 7,9 Hz, H_{arom}), 7.93 (m, 2 H, *J* = 7,9 Hz, H_{arom}), 7.55 – 7.49 (m, 4 H, H_{arom}), 7.40 (t, 2 H, *J* = 7.8 Hz, H_{arom}), 7.36 (t, 2 H, *J* = 7.8 Hz, H_{arom}), 7.32 – 7.29 (m, 3 H, H_{arom}), 5.42 – 5.37 (m, 2 H, H-2, H-3), 4.93 (d, 1 H, *J* = 9.4 Hz, H-1), 4.34 (dd, 1 H, *J* = 12.1, 2.2 Hz, H-5), 3.82 (s, 3 H, $CH_{3\text{-}COOMe}$), 2.73 (m, 1 H, H-4_{eq}), 2.03 (m, 1 H, H-4_{ax}); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 168.8 ($C{=}O_{COOMe}$), 165.7, 165.2 ($C{=}O_{Bz}$ x2), 133.30, 133.25 (CH_{arom} x2), 131.94 ($C_{q\text{-}arom}$), 129.77, 129.72 (CH_{arom} x2), 129.4, 129.1 ($C_{q\text{-}arom}$ x2), 128.9, 128.37, 128.32 (CH_{arom} x3), 86.8 (C-1), 74.0 (C-5), 72.3 (C-2), 70.5 (C-3), 52.6 ($CH_{3\text{-}COOMe}$), 33.35 (C-4); IR (neat): 2954, 1718, 1451, 1272, 1026, 906, 728, 706 cm^{-1}; HRMS Calcd for $[C_{27}H_{24}O_7S + Na]^+$: 515.1135, found 515.1130.

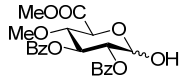


Methyl (2,3,4-tri-*O*-benzoyl-*α*/*β*-D-glucopyranose) uronate (17) To a solution of methyl (phenyl thioglucoside)uronate **8** (0.77 g, 1.3 mmol, 1.0 eq) in acetone/water 24:1 (8 mL), were *N*-bromosuccinimide (0.90 g, 5.0 mmol, 4.0 eq) added at 0 °C for one hour. The reaction was then diluted with EtOAc (100 mL) and washed with $Na_2S_2O_3$ (10% aq.) (80 mL), water (100 mL) and brine (80 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (20–40% EtOAc in petroleum ether) yielded the title compound in a 10:1 *α*/*β*-ratio as a white solid (0.14 g, 0.32 mmol, 98%). Rf = 0.35 (30% EtOAc in petroleum ether); NMR assignment for major isomer (*α*) ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 – 7.94 (m, 4 H, H_{arom}), 7.92 – 7.88 (m, 2 H, H_{arom}), 7.54 – 7.48 (m, 2 H, H_{arom}), 7.40 – 7.34 (m, 4 H, H_{arom}), 7.33 – 7.27 (m, 3 H, H_{arom}), 6.27 (t, 1 H, *J* = 9.7 Hz, H-3), 5.87 (d, 1 H, *J* = 3.5 Hz, H-1), 5.67 (t, 1 H, *J* = 9.6 Hz, H-4), 5.34 (dd, 1 H, *J* = 10.0, 3.5 Hz, H-2), 4.89 (d, 1 H, *J* = 9.8 Hz, H-5), 3.64 (s, 3 H, $CH_{3\text{-}COOMe}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.6 ($C{=}O_{COOMe}$), 165.7, 165.7, 165.4 ($C{=}O_{Bz}$ x3), 133.4, 133.4, 133.2, 129.9, 129.8, 129.7 (CH_{arom} x6), 129.0, 128.8 ($C_{q\text{-}arom}$ x2), 128.4, 128.3 (CH_{arom} x2), 90.5 (C-1), 71.6 (C-2), 70.0

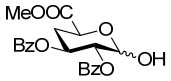
(C-4), 69.4 (C-3), 68.5 (C-5), 52.8 ($CH_{3\text{-}COOMe}$); IR (neat): 3472, 2953, 1723, 1452, 1252, 1067, 1026, 706, 687 cm^{-1}; HRMS Calcd for $[C_{28}H_{24}O_{10} + Na]^+$: 543.1262, found 543.1255.



Methyl (2,3,4-tri-*O*-benzoyl-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)-*α/β*-D-glucopyranoside) uronate (18) To a solution of 4-methoxy hemiacetal **17** (0.52 g, 1.0 mmol, 1.0 eq) in acetone (8 mL) were Cs_2CO_3 (0.49 g, 1.50 mmol, 1.5 eq) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride[39] (0.23 mL, 1.5 mmol, 1.5 eq) added at 0 °C. After 1.5 hours at 0 °C was the mixture filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–20% EtOAc in petroleum ether) yielded the title imidate as a colourless oil in a 1:3 *α/β*-ratio (0.53 g, 0.76 mmol, 76%). NMR assignment for the major isomer (*β*): R_f = 0.45 (20% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.02 – 7.94 (m, 4 H, H_{arom}), 7.92 – 7.894 (m, 2 H, H_{arom}), 7.60 – 7.51 (m, 2 H, H_{arom}), 7.47 – 7.25 (m, 8 H, H_{arom} and $H_{arom\text{-}NPh}$), 7.12 (t, 1 H, *J* = 7.8 Hz, $H_{arom\text{-}NPh}$), 6.95 (bs, 1 H, H-1), 6.40 (bd, 2 H, *J* = 6.4 Hz, $H_{arom\text{-}NPh}$), 6.26 (t, 1 H, *J* = 9.9 Hz, H-3), 5.76 (t, 1 H, *J* = 9.9 Hz, H-4), 5.76 (t, 1 H, *J* = 9.9 Hz, H-4), 5.67 (m, 1 H, H-2), 4.76 (d, 1 H, *J*= 9.9 Hz, H-5), 3.71 (s, 3 H, $CH_{3\text{-}COOMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.1 ($C{=}O_{COOMe}$), 165.5, 165.2, 165.1 ($C{=}O_{Bz}$ x3), 142.5 ($C_{q\text{-}arom\text{-}NPh}$), 133.7, 133.6, 133.6, 133.4, 129.9, 129.9 (CH_{arom} x6), 129.8, 128.7, 128.61, 128.57, 128.49, 128.46, 128.39 (C_{arom} x7), 128.35, 119.3 ($CH_{arom\text{-}NPh}$ x2), 91.8 (C-1), 70.9 (C-5), 69.9 (C-2), 69.4 (C-4), 69.2 (C-3), 53.1($CH_{3\text{-}COOMe}$).

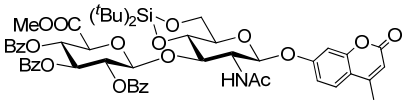


Methyl (2,3-di-*O*-benzoyl-4-*O*-methyl-*α/β*-D-glucopyranose) uronate (19) To a solution of methyl (phenyl thioglucoside)uronate **9** (0.23 g, 0.43 mmol, 1.0 eq) in DCM (8 mL), were *N*-iodosuccinimide (0.10 g, 0.45 mmol, 1.05 eq) and TFA (35 µL, 0.45 mmol, 1.05 eq) added at 0 °C. The reaction was left stirring at room temperature (2.5 hours) and was then quenched with sodium thiosulphate (20% aq.) (2.0 mL). The reaction mixture was transferred to an extraction funnel with EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (30 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (10–30% EtOAc in petroleum ether) yielded the title compound in a 10:1 *α/β*-ratio as a white solid (0.14 g, 0.32 mmol, 75%). R_f = 0.45 (30% EtOAc in petroleum ether); NMR assignment for major isomer (*α*) ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 – 8.00 (m, 2 H, H_{arom}), 7.99 – 7.95 (m, 2 H, H_{arom}), 7.56 – 7.47 (m, 2 H, H_{arom}), 7.43 – 7.33 (m, 4 H, H_{arom}), 6.00 (t, 1 H, *J* = 9.5 Hz, H-3), 5.70 (dd, 1 H, *J* = 3.7, 3.4 Hz, H-1), 5.18 (dd, 1 H, *J* = 9.5, 3.4 Hz, H-2), 4.61 (d, 1 H, *J* = 9.7 Hz, H-5), 3.88 (dd, 1 H, *J* = 9.7, 9.5 Hz, H-4), 3.83 (s, 3 H, $CH_{3\text{-}COOMe}$), 3.43 (s, 3 H, $CH_{3\text{-}OMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.5 ($C{=}O_{COOMe}$), 165.9, 165.5 ($C{=}O_{Bz}$ x2), 133.4, 133.3, 129.9, 129.7 (CH_{arom} x4), 129.5 ($C_{q\text{-}arom}$), 128.4 (CH_{arom}), 90.7 (C-1), 79.1 (C-4), 71.8 (C-2), 71.4 (C-3), 67.1 (C-5), 60.3 ($CH_{3\text{-}OMe}$), 52.8 ($CH_{3\text{-}COOMe}$); IR (neat): 3440, 2955, 2849, 1725, 1452, 1265, 1108, 1069, 709 cm^{-1}; HRMS Calcd for $[C_{22}H_{22}O_9 + Na]^+$: 453.1156, found 453.1152.



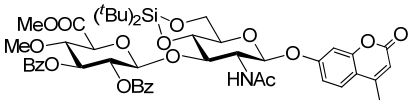
Methyl (2,3-di-*O*-benzoyl-4-deoxy-*α/β*-D-glucopyranose) uronate (20) To a solution of methyl (phenyl thioglucopyranoside)uronate **16** (0.50 g, 1.0 mmol, 1.0 eq) in DCM (8 mL), were *N*-iodosuccinimide (0.25 g, 1.1 mmol, 1.1 eq) and TFA (86 µL, 1.1 mmol, 1.1 eq) added at 0 °C. The reaction was left stirring at 0 °C (2.5 hours) and was then quenched with sodium thiosulphate (20% aq.) (10 mL). The reaction mixture was transferred to an extraction funnel with EtOAc (40 mL) and washed with water (40 mL), sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (20–40% EtOAc in petroleum ether) yielded the title compound as a white solid in a (4:1) *α/β*-mixture (0.39 g, 0.98 mmol, 96%). The *α*-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07 – 7.95 (m, 4 H, H_{arom}), 7.53 - 7.46 (m, 2 H, H_{arom}),

7.40 - 7.35 (m, 4 H, H_{arom}), 5.83 (ddd, 1 H, *J* = 11.2, 9.9, 5.2 Hz, H-3), 5.80 (bt, 1 H, *J* = 3.5 Hz, H-1), 5.31 (dd, 1 H, *J* = 9.9, 3.2 Hz, H-2), 4.89 (dd, 1 H, *J* = 12.0, 2.7 Hz, H-5), 4.42 (d, 1 H, *J* = 3.5 Hz, OH), 3.76 (s, 3 H, $CH_{3\text{-}COOMe}$), 2.75 (ddd, 1 H, *J* = 12.8, 5.0, 2.7 Hz, H-4_{eq}), 2.00 (ddd, 1 H, *J* = 12.8, 12.0, 11.2 Hz, H-4_{ax}); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.8 ($C{=}O_{COOMe}$), 165.9, 165.7 ($C{=}O_{Oac}$ x2), 133.3, 133.2, 129.8, 129.6 (CH_{arom} x4), 129.4, 129.2 ($C_{q\text{-}arom}$ x2), 128.3 (CH_{arom}), 91.2 (C-1), 72.1 (C-2), 67.8 (C-3), 66.2 (C-5), 52.5 ($CH_{3\text{-}COOMe}$), 33.1 (C-4). The *β*-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.13 – 7.95 (m, 4 H, H_{arom}), 7.53 – 7.46 (m, 2 H, H_{arom}), 7.40 - 7.35 (m, 4 H, H_{arom}), 5.48 (m, 1 H, H-3), 5.30 (m, 1 H, H-2), 4.97 (bt, 1 H, *J* = 7.2 Hz, H-1), 4.54 (bd, 1 H, *J* = 7.8 Hz, OH), 4.37 (dd, 1 H, *J* = 11.9, 2.5 Hz, H-5), 3.76 (s, 3 H, $CH_{3\text{-}COOMe}$), 2.72 (ddd, 1 H, *J* = 12.9, 5.3, 2.5 Hz, H-4_{eq}), 2.04 (m, 1 H, H-4_{ax}); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.6 ($C{=}O_{COOMe}$), 166.4, 165.7 ($C{=}O_{Oac}$ x2), 133.4, 133.3, 129.8, 129.7 (CH_{arom} x4), 129.4, 129.0 ($C_{q\text{-}arom}$ x2), 128.3 (CH_{arom}), 95.9 (C-1), 74.2 (C-2), 70.4 (C-3), 70.3 (C-5), 52.7 ($CH_{3\text{-}COOMe}$), 33.0 (C-4); IR (neat): 3440, 2956, 1718, 1451, 1261, 1069, 909, 707 cm^{-1}; HRMS Calcd for $[C_{21}H_{20}O_8 + Na]^+$: 423.1050, found 423.1048.



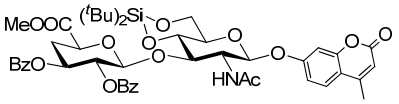
4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-*β*-D-glucopyranosyl uronate)-4,6-*O*-di-*tert*-butylsilanediyl-*β*-D-glucopyranoside (21) Method A: Hemiacetal **17** (62 mg, 0.12 mmol, 1.2 eq) and diphenyl sulfoxide (57 mg, 0.28 mmol, 2.8 eq) were coevaporated two times in anhydrous toluene, dissolved in anhydrous DCM (2 mL) and stirred with flame dried molecular sieves (3Å) for 30 minutes. The mixture was then cooled to −60 °C and Tf_2O (24 µL, 0.14 mmol, 1.44 eq) was added. The reaction mixture was heated to −20 °C (or the temperature stated in Table 1, this chapter) and left stirring at this temperature for 1 hour. *N*-Acetylglucosamine acceptor **5** (52 mg, 0.1 mmol, 1.0 eq) was coevaporated two times in anhydrous toluene (with a drop of anhydrous DCM) and then dissolved in anhydrous DCM (1.5 mL) before addition to the activated donor. The reaction was warmed to ~0 °C and left stirring at this temperature over night. The reaction was then quenched with triethylamine (0.07 mL, 0.5 mmol, 5.0 eq), transferred to an extraction funnel using EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqeous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (10–40% EtOAc in DCM) produced the title compound as a white solid (76 mg, 0.074 mmol, 74%). **Method B:** Imidate donor **18** (72 mg, 0.12 mmol, 1.2 eq) and *N*-Acetylglucosamine acceptor **5** (52 mg, 0.10 mmol, 1.0 eq) were coevaporated two times in anhydrous toluene, dissolved in anhydrous DCM (3 mL). The mixture was then cooled to 0 °C and triflic acid (~2 µL, 20 µmol, 0.2 eq) was added. The reaction was then stirred at this temperature until TLC showed full conversion of starting imidate (~3 h). The reaction was then transferred to an extraction funnel with EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqeous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (10–40% EtOAc in DCM) produced the title compound as a white solid (49 mg, 0.053 mmol, 53%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.97 – 7.93 (m, 4 H, H_{arom}), 7.86 - 7.82 (m, 2 H, H_{arom}), 7.55 - 7.51 (m, 2 H, H_{arom}), 7.47 –7.42 (m, 2 H, H-$5_{4\text{-}MU}$ and H_{arom}), 7.41 - 7.37 (m, 4 H, H_{arom}), 7.31 - 7.27 (m, 2 H, H_{arom}), 6.88 – 6.85 (m, 2 H, H-$6_{4\text{-}MU}$ and H-$8_{4\text{-}MU}$), 6.14 (d, 1 H, *J* = 1.3 Hz, H-$3_{4\text{-}MU}$), 5.95 (d, 1 H, *J* = 7.0 Hz, H-1), 5.88 (t, 1 H, *J* = 9.1 Hz, H-3′), 5.87 (d, 1 H, *J* = 7.6 Hz, NH), 5.72 (t, 1 H, *J* = 9.4 Hz, H-4′), 5.56 (dd, 1 H, *J* = 9.1, 7.6, 7.2 Hz, H-2′), 5.30 (d, 1 H, *J* = 7.2 Hz, H-1′), 4.55 (dd, 1 H, *J* = 10.1, 8.5 Hz, H-3), 4.34 (d, 1 H, *J* = 9.5 Hz, C-5′), 4.19 (dd, 1 H, *J* = 10.3, 5.0 Hz, C-6_{eq}), 4.06 (t, 1 H, *J* = 9.0 Hz, H-4), 3.92 (t, 1 H, *J* = 10.3 Hz, C-6_{ax}), 3.69 – 363 (m, 4 H, C-5 and $CH_{3\text{-}COOMe}$), 3.36 (m, 1 H, H-2), 2.36 (d, 3 H, *J* = 1.3 Hz, $CH_{3\text{-}4MU}$), 1.62 (s, 3 H, $CH_{3\text{-}NAc}$), 1.04 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.01 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 171.1 ($C{=}O_{NAc}$), 167.4 ($C{=}O_{COOMe}$), 165.6, 165.2, 165.0 ($C{=}O_{OBz}$ x3), 160.9, 159.5, 154.7, 152.2 (C-$2_{4\text{-}MU}$, C-$7_{4\text{-}MU}$, C-$9_{4\text{-}MU}$ and C-$10_{4\text{-}MU}$), 133.6, 133.5, 133.3, 129.8, 129.8, 129.4 (CH_{arom}

x6), 128.9, 128.4, 128.6 ($C_{q\text{-arom}}$ x3), 128.5, 128.4, 128.3 (CH_{arom} x3), 125.6 (C-$5_{4\text{-MU}}$), 115.2 (C-$4_{4\text{-MU}}$), 113.4 (C-$6_{4\text{-MU}}$), 112.9 (C-$3_{4\text{-MU}}$), 104.4 (C-$8_{4\text{-MU}}$), 99.8 (C-1′), 96.8 (C-1), 80.1 (C-3), 76.1 (C-4), 73.0 (C-5′), 72.8 (C-2′), 72.3 (C-3′), 70.4 (C-5), 70.1 (C-4′), 66.1 (C-6), 57.8 (C-2), 52.8 ($CH_{3\text{-COOMe}}$), 27.3, 27.0 ($CH_{3\text{-tBu-Si}}$ x2), 23.2 ($CH_{3\text{-NAc}}$), 22.5, 19.9 ($C_{q\text{-tBu-Si}}$ x2), 18.6 ($CH_{3\text{-4MU}}$); IR (neat): 2934, 2860, 1734, 1616, 1262, 1068, 1026, 828, 709 cm^{-1}; HRMS Calcd for $[C_{54}H_{59}NO_{17}Si + Na]^+$: 1044.3444, found 1044.3449.



4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-methyl-*β*-D-glucopyranosyl uronate)-4,6-*O*-di-*tert*-butylsilanediyl-*β*-D-glucopyranoside (23)

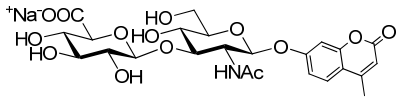
Hemiacetal **19** (0.14 g, 0.32 mmol, 1.2 eq) and diphenyl sulfoxide (0.15 g, 0.75 mmol, 2.8 eq) were coevaporated two times in anhydrous toluene, dissolved in anhydrous DCM (6 mL) and stirred with flame dried molecular sieves (3Å) for 30 minutes. The mixture was then cooled to −60 °C and Tf_2O (65 µL, 0.39 mmol, 1.44 eq) was added. The reaction mixture was heated to −20 °C and left stirring at this temp for one hour. *N*-Acetylglucosamine acceptor **5** (0.14 g, 0.27 mmol, 1.0 eq) was coevaporated two times in anhydrous toluene (with a drop of anhydrous DCM) and then dissolved in anhydrous DCM (3 mL) before addition to the activated donor. The reaction was warmed to ~0 °C and left stirring at this temperature over night. The reaction was then quenched with triethylamine (0.19 mL, 1.35 mmol, 5.0 eq), transferred to an extraction funnel using EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqeous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (10–50% EtOAc in petroleum ether) produced the title compound as a white solid (0.13 g, 0.14 mmol, 53%). Rf = 0.54 (30% EtOAc in DCM); $[\alpha]_D^{22}$: +45 (C = 1.0 $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.98 – 7.93 (m, 4 H, H_{arom}), 7.56 - 7.50 (m, 2 H, H_{arom}), 7.44 (d, 1 H, *J* = 8.3 Hz, H-$5_{4\text{-MU}}$), 7.42 - 7.36 (m, 4 H, H_{arom}), 6.84 (dd, 1 H, *J* = 8.3, 2.3 Hz, H-$6_{4\text{-MU}}$), 6.82 (d, 1 H, *J* = 2.3 Hz, H-$8_{4\text{-MU}}$), 6.13 (d, 1 H, *J* = 1.1 Hz, H-$3_{4\text{-MU}}$), 5.74 (d, 1 H, *J* = 8.2 Hz, H-1), 5.68 (d, 1 H, *J* = 7.1 Hz, NH), 5.60 (t, 1 H, *J* = 9.1 Hz, H-3′), 5.40 (dd, 1 H, *J* = 9.1, 7.5 Hz, H-2′), 5.11 (d, 1 H, *J* = 7.4 Hz, H-1′), 4.41 (dd, 1 H, *J* = 9.9, 8.6 Hz, H-3), 4.18 (dd, 1 H, *J* = 10.3, 5.0 Hz, H-6_{eq}), 4.10 (d, 1 H, *J* = 9.1 Hz, C-5′), 4.00 – 3.86 (m, 3 H, C-4, C-6_{ax} and C-4′), 3.80 (s, 3 H, $CH_{3\text{-COOMe}}$), 3.61 (ddd, 1 H, *J* = 9.9, 9.7, 5.0 Hz, H-5), 3.39 (s, 3 H, $CH_{3\text{-OMe}}$), 3.30 (ddd, 1 H, *J* = 9.9, 8.2, 7.1 Hz, H-2), 2.36 (d, 3 H, *J* = 1.1 Hz, $CH_{3\text{-4MU}}$), 1.64 (s, 3 H, $CH_{3\text{-NAc}}$), 1.06 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 1.01 (s, 9 H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.9 ($C{=}O_{NAc}$), 168.5 ($C{=}O_{COOMe}$), 165.4, 165.2 ($C{=}O_{OBz}$ x2), 160.9, 159.5, 154.6, 152.3 (C-$2_{4\text{-MU}}$, C-$7_{4\text{-MU}}$, C-$9_{4\text{-MU}}$ and C-$10_{4\text{-MU}}$), 133.6, 133.3, 129.7, 129.7 (CH_{arom} x4), 129.1, 129.0 ($C_{q\text{-arom}}$ x2), 128.6, 128.4 (CH_{arom} x2), 125.6 (C-$5_{4\text{-MU}}$), 115.2 (C-$4_{4\text{-MU}}$), 113.4 (C-$6_{4\text{-MU}}$), 112.8 (C-$3_{4\text{-MU}}$), 104.3 (C-$8_{4\text{-MU}}$), 100.6 (C-1′), 96.7 (C-1), 80.1 (C-3), 79.1 (C-4′), 76.1 (C-4), 74.4 (C-5′), 74.2 (C-3′), 73.0 (C-2′), 70.4 (C-5), 66.1 (C-6), 60.3 ($CH_{3\text{-OMe}}$), 57.7 (C-2), 52.6 ($CH_{3\text{-COOMe}}$), 27.3, 26.9 ($CH_{3\text{-tBu-Si}}$ x2), 23.2 ($CH_{3\text{-NAc}}$), 22.5, 19.9 ($C_{q\text{-tBu-Si}}$ x2), 18.6 ($CH_{3\text{-4MU}}$); IR (neat): 2936, 2860, 1734, 1616, 1390, 1272, 1090, 832, 709 cm^{-1}; HRMS Calcd for $[C_{48}H_{57}NO_{16}Si + Na]^+$: 954.3339, found 954.3345.



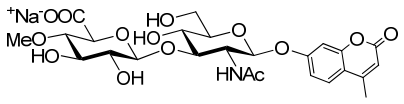
4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-deoxy-*β*-D-glucopyranosyl uronate)-4,6-*O*-di-*tert*-butylsilanediyl-*β*-D-glucopyranoside (24)

Hemiacetal **20** (0.20 g, 0.50 mmol, 1.2 eq) and diphenyl sulfoxide (0.23 g, 1.15 mmol, 2.8 eq) were coevaporated two times in anhydrous toluene, dissolved in anhydrous DCM (10 mL) and stirred with flame dried molecular sieves (3Å) for 30 minutes. The mixture was then cooled to −60 °C and Tf_2O (0.10 mL, 0.62 mmol, 1.44 eq) was added. The reaction mixture was heated to −20 °C and left stirring at this temp for one hour. *N*-Acetylglucosamine acceptor **5** (0.21 g,

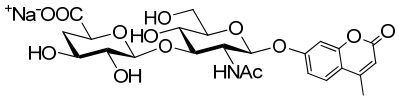
0.41 mmol, 1.0 eq) was coevaporated two times in anhydrous toluene (with a drop of anhydrous DCM) and then dissolved in anhydrous DCM (5 mL) before addition to the activated donor. The reaction was warmed to ~0 °C and left stirring at this temperature over night. The reaction was then quenched with triethylamine (0.29 mL, 2.1 mmol, 5.0 eq), transferred to an extraction funnel using EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqeous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (10–50% EtOAc in petroleum ether) produced the title compound as a white solid (0.30 g, 0.34 mmol, 82%). Rf = 0.50 (30% EtOAc in DCM); $[\alpha]_D^{22}$: +61 (C = 0.5 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 – 7.96 (m, 2 H, H_{arom}), 7.94 - 7.89 (m, 2 H, H_{arom}), 7.53 - 7.48 (m, 2 H, H_{arom}), 7.43 (d, 1 H, *J* = 8.5 Hz, H-5$_{4\text{-MU}}$), 7.41 - 7.34 (m, 4 H, H_{arom}), 6.85 (dd, 1 H, *J* = 8.5, 2.4 Hz, H-6$_{4\text{-MU}}$), 6.84 (d, 1 H, *J* = 2.4 Hz, H-8$_{4\text{-MU}}$), 6.12 (d, 1 H, *J* = 1.1 Hz, H-3$_{4\text{-MU}}$), 5.98 (d, 1 H, *J* = 7.2 Hz, NH), 5.82 (d, 1 H, *J* = 8.2 Hz, H-1), 5.44 (dd, 1 H, *J* = 9.4, 7.5 Hz, H-2′), 5.34 (ddd, 1 H, *J* = 11.4, 9.4, 5.2 Hz, H-3′), 5.20 (d, 1 H, *J* = 7.5 Hz, H-1′), 4.45 (dd, 1 H, *J* = 9.8, 8.8 Hz, H-3), 4.31 (dd, 1 H, *J* = 12.1, 2.2 Hz, C-5′), 4.19 (dd, 1 H, *J* = 10.3, 4.9 Hz, C-6$_{eq}$), 4.10 (t, 1 H, *J* = 9.0 Hz, H-4), 3.93 (dd, 1 H, *J* = 10.3, 9.8 Hz, H-6$_{ax}$), 3.79 (s, 3 H, $CH_{3\text{-COOMe}}$), 3.65 (ddd, 1 H, *J* = 9.8, 9.0, 4.9 Hz, H-5), 3.47 (ddd, 1 H, *J* = 9.8, 8.2, 7.2 Hz, H-2), 2.70 (ddd, 1 H, *J* = 12.6, 5.2, 2.2 Hz, H-4′$_{eq}$), 2.35 (d, 3 H, *J* = 1.1 Hz, $CH_{3\text{-4MU}}$), 2.05 (ddd, 1 H, *J* = 12.6, 12.1, 11.4 Hz, H-4′$_{ax}$), 1.56 (s, 3 H, $CH_{3\text{-NAc}}$), 1.05 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 1.03 (s, 9 H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 170.8 ($C{=}O_{NAc}$), 169.2 ($C{=}O_{COOMe}$), 165.7, 165.4 ($C{=}O_{OBz}$ x2), 160.9, 159.6, 154.6, 152.5 (C-2$_{4\text{-MU}}$, C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 133.4, 133.3, 129.7, 129.6 (CH_{arom} x4), 129.2, 129.0 ($C_{q\text{-arom}}$ x2), 128.5, 128.3 (CH_{arom} x2), 125.5 (C-5$_{4\text{-MU}}$), 115.1 (C-4$_{4\text{-MU}}$), 113.5 (C-6$_{4\text{-MU}}$), 112.4 (C-3$_{4\text{-MU}}$), 104.2 (C-8$_{4\text{-MU}}$), 99.4 (C-1′), 97.0 (C-1), 79.7 (C-3), 76.4 (C-4), 73.3 (C-2′), 71.4 (C-3′), 70.6 (C-5), 70.3 (C-5′), 66.0 (C-6), 57.4 (C-2), 52.5 ($CH_{3\text{-COOMe}}$), 33.2 (C-4′), 27.3, 26.9 ($CH_{3\text{-tBu-Si}}$ x2), 23.0 ($CH_{3\text{-NAc}}$), 22.5, 19.9 ($C_{q\text{-tBu-Si}}$ x2), 18.6 ($CH_{3\text{-4MU}}$); HRMS Calcd for $[C_{47}H_{55}NO_{15}Si + Na]^+$: 924.3233, found 924.3237.



4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(*β*-D-glucopyranosyl uronicacid)-*β*-D-glucopyranoside sodium salt (25) Protected dimer **21** (80 mg, 78 µmol, 1.0 eq) was dissolved in anhydrous methanol (3 mL) and sodium methoxide (30% in MeOH) (11 µL, 78 µmol, 1.0 eq) was added under an atmosphere of argon. The reaction was run over night at ambient temperature and monitored by HPLC-MS. The reaction was quenched with AcOH (0.2 mL) and was then coevaporated with toluene. The residue was dissolved in pyridine (2 mL) and HF·Et_3N (25µL, 0.16 mmol, 2.0 eq) was added. The reaction was run for 6 hours at ambient temperature, monitored by HPLC-MS. Upon completion water (2 mL) and sat. aq. Na_2CO_3 (1 mL) were added and the mixture stirred over night at ambient temperature. The reaction was monitored by HPLC-MS and was then concentrated *in vacuo*. The residue was purified by HPLC according to the general procedure. Repeated lyophilization followed by filtration over wet Amberlite-Na^+ (2 mL) gave the title compound as a white solid (32 mg, 56 µmol, 73%). ^{1}H NMR (500 MHz, D_2O) δ: 7.42 (d, 1H, *J* = 8.9 Hz, H-5$_{4\text{-MU}}$), 6.89 (dd, 1H, *J* = 8.8, 2.4 Hz, H-6$_{4\text{-MU}}$), 6.75 (d, 1H, *J* = 2.3 Hz, H-8$_{4\text{-MU}}$), 5.98 (d, 1H, *J* = 1.3 Hz, H-3$_{4\text{-MU}}$), 5.22 (d, 1H, *J* = 8.5 Hz, H-1), 4.57 (d, 1H, *J* = 7.8 Hz, H-1′), 4.17 (dd, 1H, *J* = 10.2, 8.7 Hz, H-2), 3.97 (dd, 1H, *J* = 12.6, 2.0 Hz, H-6$_a$), 3.92 (dd, 1H, *J* = 10.2, 8.1 Hz, H-3), 3.85 (dd, 1H, *J* = 12.6, 4.3 Hz, H-6$_b$), 3.78 (m, 1H, H-5′), 3.73 – 3.65 (m, 2H, H-5 and H-4), 3.56 – 3.49 (m, 2H, H-4′ and H-3′), 3.38 (m, 1H, H-2′), 2.23 (d, 3H, *J* = 1.3 Hz, $CH_{3\text{-4MU}}$), 2.06 (s, 3H, $CH_{3\text{-NAc}}$). ^{13}C NMR (126 MHz, D_2O) δ: 176.7 ($C{=}O_{COO\text{-}Na+}$), 176.3 ($C{=}O_{NAc}$), 165.4, 160.7, 157.2, 154.9 (C-2$_{4\text{-MU}}$, C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 127.9 (C-5$_{4\text{-MU}}$), 116.4 (C-4$_{4\text{-MU}}$), 115.2 (C-6$_{4\text{-MU}}$), 112.6 (C-3$_{4\text{-MU}}$), 104.9 (C-8$_{4\text{-MU}}$), 104.3 (C-1′), 99.9 (C-1), 83.7 (C-3), 77.2 (C-5), 77.1 (C-5′), 76.8 (C-3′), 74.2 (C-2′), 73.1 (C-4′), 69.8 (C-4), 61.9 (C-6), 55.8 (C-2), 23.8 ($CH_{3\text{-4MU}}$), 19.3 ($CH_{3\text{-NAc}}$); ESI-MS *m/z*: 556.2 $[M + H]^+$.



4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(4-*O*-methyl-*β*-D-glucopyranosyl uronicacid)-*β*-D-glucopyranoside sodium salt (26) Protected dimer **23** (48 mg, 51 µmol, 1.0 eq) was dissolved in anhydrous methanol (2 mL) and sodium methoxide (30% in MeOH) (7 µL, 51 µmol, 1.0 eq) was added under an atmosphere of argon. The reaction was run over night at ambient temperature and monitored by HPLC-MS. The reaction was quenched with AcOH (0.2 mL) and was then coevaporated with toluene. The residue was dissolved in pyridine (2 mL) and HF·Et_3N (17 µL, 0.10 mmol, 2.0 eq) was added. The reaction was run for 6 hours at ambient temperature, monitored by HPLC-MS. Upon completion water (2 mL) and sat. aq. Na_2CO_3 (1 mL) were added and the mixture stirred over night at ambient temperature. The reaction was monitored by HPLC-MS and was then concentrated *in vacuo*. The residue was purified py HPLC according to the general procedure. Repeated lyophilization followed by filtration over wet Amberlite-Na^+ (2 mL) gave the title compound as a white solid (19 mg, 32 µmol, 63%). ^{1}H NMR (500 MHz, MeOD-d_4) δ: 7.72 (d, 1 H, *J* = 8.7 Hz, H-5$_{4\text{-MU}}$), 7.07 – 7.02 (m, 2 H, H-6$_{4\text{-MU}}$ and H-8$_{4\text{-MU}}$), 6.00 (d, 1 H, *J* = 1.2 Hz, H-3$_{4\text{-MU}}$), 5.24 (d, 1 H, *J* = 8.5 Hz, H-1), 4.38 (d, 1 H, *J* = 7.6 Hz, H-1′), 4.10 (dd, 1 H, *J* = 10.3, 8.5 Hz, H-2), 3.94 (dd, 1 H, *J* = 12.2, 1.8 Hz, H-6$_a$), 3.81 (dd, 1 H, *J* = 10.3, 7.9 Hz, H-3), 3.76 (dd, 1 H, *J* = 12.2, 4.8 Hz, H-6$_b$), 3.66 (d, 1 H, *J* = 9.6 Hz, H-5′), 3.58 –3.51 (m, 5 H, H-4, H-5 and $CH_{3\text{-OMe}}$), 3.45 (t, 1 H, *J* = 9.0 Hz, H-3′), 3.36 (dd, 1 H, *J* = 9.2, 7.6 Hz, H-2′), 3.28 (t, 1 H, *J* = 9.0 Hz, H-4′), 2.46 (d, 3 H, *J* = 1.2 Hz, $CH_{3\text{-4-MU}}$), 2.00 (s, 3 H, $CH_{3\text{-NAc}}$). ^{13}C NMR (126 MHz, MeOD-d_4) δ: 176.1 (C=O$_{\text{COO-Na+}}$), 174.5 (C=O$_{\text{NAc}}$), 163.2, 161.8, 156.0, 155.4 (C-2$_{4\text{-MU}}$, C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 127.4 (C-5$_{4\text{-MU}}$), 116.2 (C-4$_{4\text{-MU}}$), 114.9 (C-6$_{4\text{-MU}}$), 113.0 (C-3$_{4\text{-MU}}$), 104.9 (C-8$_{4\text{-MU}}$), 104.8 (C-1′), 100.1 (C-1), 83.93 (C-3), 83.85 (C-4′), 78.2 (C-5′), 78.1 (C-5), 77.2 (C-3′), 74.4 (C-2′), 70.3 (C-4), 62.4 (C-6), 60.7 ($CH_{3\text{-OMe}}$), 55.9 (C-2), 23.2 ($CH_{3\text{-4MU}}$), 18.6 ($CH_{3\text{-NAc}}$); HRMS Calcd for [$C_{25}H_{31}NO_{14}$ + Na]$^+$: 592.1637, found 592.1634.



4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(4-deoxy-*β*-D-glucopyranosyl uronicacid)-*β*-D-glucopyranoside sodium salt (27) Protected dimer **24** (0.13 mg, 0.14 mmol, 1.0 eq) was dissolved in anhydrous methanol (4 mL) and sodium methoxide (30% in MeOH) (40 µL, 0.14 mmol, 1.0 eq) was added under an atmosphere of argon. The reaction was run over night at ambient temperature and monitored by HPLC-MS. The reaction was quenched with AcOH (0.3 mL) and was then coevaporated with toluene. The residue was dissolved in pyridine (2 mL) and HF·Et_3N (47 µL, 0.29 mmol, 2.0 eq) was added. The reaction was run for 6 hours at ambient temperature, monitored by HPLC-MS. Upon completion water (2 mL) and sat. aq. Na_2CO_3 (1 mL) were added and the mixture stirred over night at ambient temperature. The reaction was monitored by HPLC-MS and was then concentrated *in vacuo*. The residue was purified py HPLC according to the general procedure. Repeated lyophilization followed by filtration over wet Amberlite-Na^+ (3 mL) gave the title compound as a white solid (62 mg, 0.11 mmol, 77%). ^{1}H NMR (500 MHz, D_2O) δ: 7.42 (d, 1H, *J* = 8.9 Hz, H-5$_{4\text{-MU}}$), 6.88 (dd, 1H, *J* = 8.8, 2.1 Hz, H-6$_{4\text{-MU}}$), 6.79 (d, 1H, *J* = 2.3 Hz, H-8$_{4\text{-MU}}$), 6.00 (s, 1H, H-3$_{4\text{-MU}}$), 5.20 (d, 1H, *J* = 8.5 Hz, H-1), 4.41 (d, 1H, *J* = 7.8 Hz, H-1′), 4.11 (dd, 1H, *J* = 10.4, 8.5 Hz, H-2), 3.98 (dd, 1H, *J* = 12.2, 2.2 Hz, H-5′), 3.92 (dd, 1H, *J* = 12.6, 1.7 Hz, H-6$_a$), 3.84 (dd, 1H, *J* = 10.5, 7.8 Hz, H-3), 3.79 (m, 1H, H-6$_b$), 3.71 (m, 1 H, H-5), 3.63 (m, 1H, H-4), 3.20 (dd, 1 H, *J* = 9.2, 7.9 Hz, H-2′), 2.29 – 2.21 (m, 4 H, H-4$_{eq}$′ and $CH_{3\text{-4MU}}$), 1.99 (s, 3H, $CH_{3\text{-NAc}}$), 1.52 (q, 1 H, *J* = 12.2 Hz, H-4$_{ax}$′). ^{13}C NMR (126 MHz, D_2O) δ: 178.3 (C=O$_{\text{COO-Na+)}}$, 176.2 (C=O$_{\text{NAc}}$), 165.4, 160.6, 157.2, 154.9 (C-2$_{4\text{-MU}}$, C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 127.9 (C-5$_{4\text{-MU}}$), 116.4 (C-4$_{4\text{-MU}}$), 115.1 (C-6$_{4\text{-MU}}$), 112.6 (C-3$_{4\text{-MU}}$), 104.9 (C-8$_{4\text{-MU}}$), 104.4 (C-1′), 99.8 (C-1), 83.9 (C-3), 77.1 (C-5), 75.7 (C-2′), 73.7 (C-5′), 71.7 (C-3′), 69.8 (C-4), 61.8 (C-6), 55.6 (C-2), 37.5 (C-4′), 23.6 ($CH_{3\text{-4MU}}$), 19.2 ($CH_{3\text{-NAc}}$); ESI-MS *m/z*: 540.2 [M + H]$^+$, 562.2 [M + Na]$^+$.

References and footnotes

1. Gandhi, N. S.; Mancera, R. L. *Chem. Biol. Drug Des.* **2008,** *72* (6), 455-482.
2. Volpi, N.; Schiller, J.; Stern, R.; Soltes, L. *Curr. Med. Chem.* **2009,** *16* (14), 1718-1745.
3. Stern, R.; Asari, A. A.; Sugahara, K. N. *Eur. J. Cell Biol.* **2006,** *85* (8), 699-715.
4. Jiang, D.; Liang, J.; Noble, P. W. *Annu. Rev. Cell Dev. Biol.* **2007,** *23*, 435-461.
5. Deed, R.; Rooney, P.; Kumar, P.; Norton, J. D.; Smith, J.; Freemont, A. J.; Kumar, S. *Int. J. Cancer* **1997,** *71* (2), 251-256.
6. Delmage, J. M.; Powars, D. R.; Jaynes, P. K.; Allerton, S. E. *Ann. Clin. Lab. Sci.* **1986,** *16* (4), 303-310.
7. Forrester, J. V.; Balazs, E. A. *Immunology* **1980,** *40* (3), 435-446.
8. Noble, P. W. *Matrix Biol.* **2002,** *21* (1), 25-29.
9. Slevin, M.; Kumar, S.; Gaffney, J. *J. Biol. Chem.* **2002,** *277* (43), 41046-41059.
10. Sugahara, K. N.; Hirata, T.; Hayasaka, H.; Stern, R.; Murai, T.; Miyasaka, M. *J. Biol. Chem.* **2006,** *281* (9), 5861-5868.
11. Chai, S.; Chai, Q.; Danielsen, C. C.; Hjorth, P.; Nyengaard, J. R.; Ledet, T.; Yamaguchi, Y.; Rasmussen, L. M.; Wogensen, L. *Circ. Res.* **2005,** *96* (5), 583-591.
12. Cuff, C. A.; Kothapalli, D.; Azonobi, I.; Chun, S.; Zhang, Y.; Belkin, R.; Yeh, C.; Secreto, A.; Assoian, R. K.; Rader, D. J.; Pura, E. *J. Clin. Invest.* **2001,** *108* (7), 1031-1040.
13. Bot, P. T.; Hoefer, I. E.; Piek, J. J.; Pasterkamp, G. *Curr. Med. Chem.* **2008,** *15* (8), 786-791.
14. Stern, R. *Eur. J. Cell Biol.* **2004,** *83* (7), 317-325.
15. Jacobsson, M.; Malmberg, J.; Ellervik, U. *Carbohydr. Res.* **2006,** *341* (10), 1266-1281.
16. Horton, D. *Org. Synt. Coll. Vol.* **1973,** *5*, 1.
17. Inaba, T.; Ohgushi, T.; Iga, Y.; Hasegawa, E. *Chem. Pharm. Bull.* **1984,** *32* (4), 1597-1603.
18. Kur'yanov, V. O.; Chupakhina, T. A.; Zemlyakov, A. E.; Kotlyar, S. A.; Kamalov, G. L.; Chirva, V. Y. *Rus. J. Bioorg. Chem.* **2001,** *27* (6), 385-389.
19. Huitink, G. M. *Talanta* **1988,** *35* (12), 973-976.
20. The 4,6-silylene protective group was chosen over the more common 4,6-benzylidene, due to increased acid-resistance and solubility, resulting in improved acceptor properties.
21. Corey, E. J.; Hopkins, P. B. *Tetrahedron Lett.* **1982,** *23* (47), 4871-4874.
22. Ziegler, T.; Eckhardt, E.; Herold, G. *Liebigs Ann. Chem.* **1992,** *1992* (5), 441-451.
23. van den Bos, L. J.; Codée, J. D. C.; van der Toorn, J. C.; Boltje, T. J.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2004,** *6* (13), 2165-2168.
24. Walker, H. G.; Gee, M.; McCready, R. M. *J. Org. Chem.* **1962,** *27* (6), 2100-2102.
25. Kühnel, E.; Laffan, D.; Lloyd-Jones, G.; Martínez del Campo, T.; Shepperson, I.; Slaughter, J. *Angew. Chem., Int. Ed.* **2007,** *46* (37), 7075-7078.
26. Lindley, M. G.; Birch, G. G.; Khan, R. *Carbohydr. Res.* **1975,** *43* (2), 360-365.
27. Madaj, J.; Trynda, A.; Jankowska, M.; Wisniewski, A. *Carbohydr. Res.* **2002,** *337* (16), 1495-1498.
28. Kobayashi, S.; Morii, H.; Itoh, R.; Kimura, S.; Ohmae, M. *J. Am. Chem. Soc.* **2001,** *123* (47), 11825-11826.
29. Kondo, H.; Aoki, S.; Ichikawa, Y.; Halcomb, R. L.; Ritzen, H.; Wong, C.-H. *J. Org. Chem.* **1994,** *59* (4), 864-877.
30. Dinkelaar, J.; Witte, M. D.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Carbohydr. Res.* **2006,** *341* (10), 1723-1729.
31. Motawia, M. S.; Marcussen, J.; Møller, B. L. *J. Carbohydr. Chem.* **1995,** *14* (9), 1279 - 1294.
32. Yu, B.; Tao, H. *Tetrahedron Lett.* **2001,** *42* (12), 2405-2407.
33. Codée, J. D. C.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003,** *5* (9), 1519-1522.

34. Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001,** *123* (37), 9015-9020.
35. Orthoesters can be rearranged to form a normal glycosidic linkage under the influence of catalytic amount of acids. This was however unsuccessful with orthoester **21**.
36. Garcia, B. A.; Poole, J. L.; Gin, D. Y. *J. Am. Chem. Soc.* **1997,** *119* (32), 7597-7598.
37. This stands in slight contrast to the use of a trichloroimidate uronic acid donor that failed in a condensation with a GlcNAc acceptor, resulting in intermolecular acetylation of the GlcNAc C3-OH. See ref. 26.
38. Noteworthy, is that any other deprotection sequence failed due to formation of poorly soluble intermediates. This was exemplified by the formation of a gel when the silylene protective group was removed first.
39. Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K. *J. Org. Chem.* **1993,** *58* (1), 32-35.

Chapter 5

Synthesis of Fluorogenic Hyaluronan Tetramers as Hyaluronidase Probes

Introduction

Glycosides bearing a 4-methylumbelliferyl moiety have been successfully utilized for the monitoring of the activity of many different glycosidases. For instance, hexosaminidase activity has been investigated using a 4-methylumbelliferyl *N*-acetyl-*β*-D-glucosaminide substrate in Tay-Sachs patients.[1] In **Chapter 4**, the synthesis of three dimeric hyaluronidase probes, bearing an anomeric 4-methylumbelliferyl (4MU) fluorophore was described. These probes were not cleaved by the two mammalian hyaluronidases bovine PH20 or human Hyal2, but were successfully processed by a combination of two other hyaluronic acid-degrading enzymes, namely *β*-*exo*-glucuronidase and *exo*-*β*-*N*-acetylglucosaminidase, which are present in the human spleen. This indicates the viability of the dimeric hyaluronic acid probes as substrates for (*exo*-)glycosidases. Mammalian hyaluronidases on the other hand, are *endo*-glycosidases that may require larger hyaluronic acid fragments for adequate recognition and binding affinity. The smallest fragments produced by the mammalian

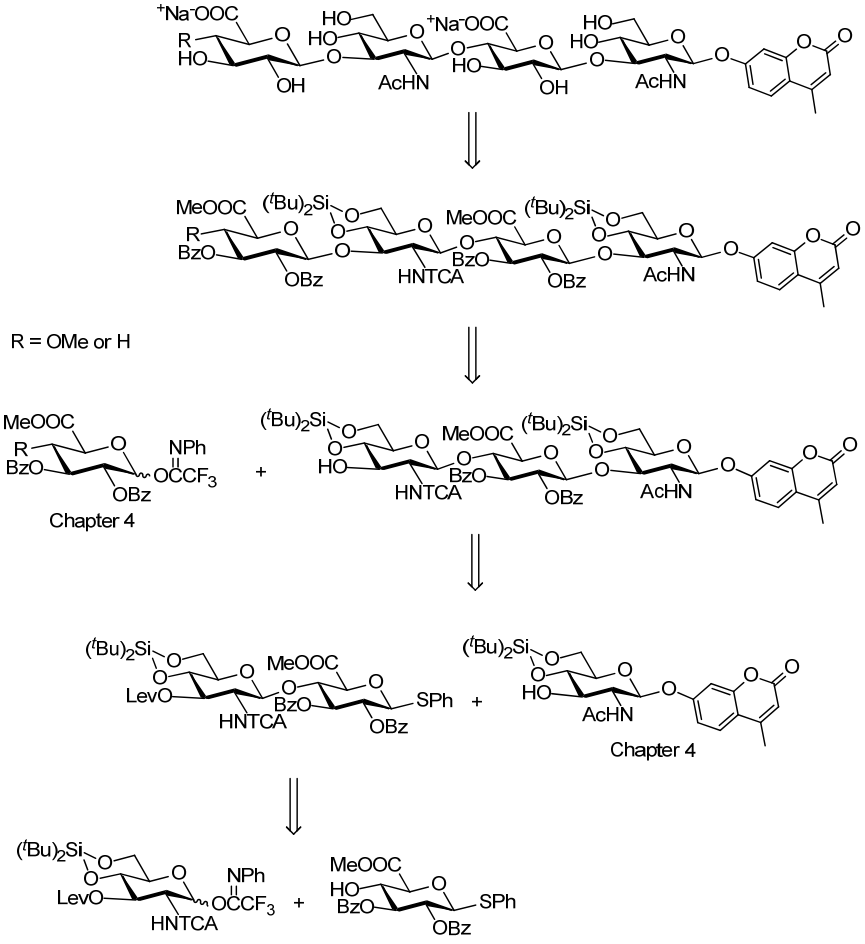
hyaluronidases are tetramers,[2] indicating that hyaluronic acid tetramers bearing a 4MU aglycon, can serve as potential hyaluronidase probes.

This chapter describes the synthesis and evaluation of two hyaluronic acid C4'''-modified tetramers **22** and **23**, which bear an anomeric 4-methylumbelliferyl group for fluorometric read-out in a continuous hyaluronidase activity assay.

Results and Discussion

Two hyaluronic acid tetramers equipped with an anomeric 4MU and either deoxygenated or methylated at the C4'''-position, were devised as target structures. Taking advantage of the study presented in **Chapter 4**, it was decided to use the two available C4-capped *β*-D-glucuronic acid donors and the *N*-actyl-*β*-D-glucosamine-4MU acceptor for the synthesis of the protected tetrameric hyaluronan probes. A retrosynthetic analysis of the assembly of the target hyaluronidase probes is presented in Scheme 1.

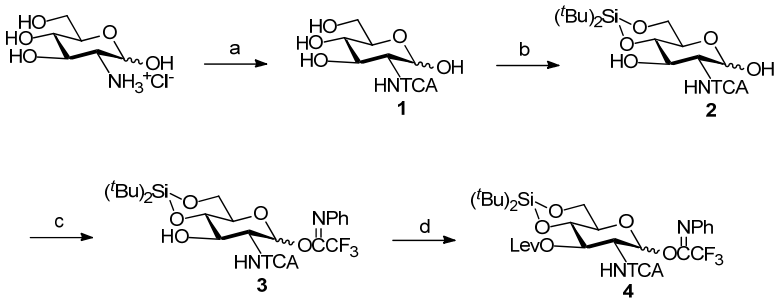
Scheme 1. Retrosynthetic analysis of the formation of two tetrameric hyaluronidase probes.



Chapter 4 described the use of an *N*-acetyl glucosamine acceptor for the assembly of the GlcA-(*β*-1,3)-GlcNAc glycosidic linkage. Condensation reactions utilizing *N*-acetyl-D-glucosamine donors are notoriously inefficient, particularly with acceptors that are poor nucleophiles such as glucuronic acids.[3] A number of investigations have been published, which utilize *N*-protected glucosamines for the synthesis of hyaluronic acid oligomers. These include *N*-phthalimido (*N*-Phth),[4-6] *N*-trichloroethyloxycarbonyl (*N*-Troc)[7] or *N*-trichloroactyl (*N*-TCA).[8-10]

The protective group pattern in dimer **6** (Scheme 3) was selected on the basis of a previous synthesis of hyaluronic acid oligomers.[10] The *N*-TCA protective group can be removed under basic conditions (KOH/EtOH) forming the free amine,[10] or converted to the *N*-acetyl derivative directly by either radical mediated reductive dechlorination using the tributylstannene/AIBN reagent couple[11] or by reduction of the chlorides with zinc/acetic acid.[12,13] Deprotection of the *N*-TCA protecting group in the final tetramers with nucleophilic reagents such as KOH, may result in unwanted opening of the coumarine lactone functionality. Thus, transformation of the *N*-TCA into the *N*-acetyl products, using either radical or reductive procedures presents the most attractive alternative.

Scheme 2. Synthesis of D-glucosamine donor **4**.



Reagents and conditions: [a] TCA-Cl, Et_3N, methanol r.t., 3 days, 51%; [b] $(^tBu)_2SiOTf_2$, pyridine, DMF, −40 °C, 45 min, 93%; [c] ClC(NPh)CF_3, Cs_2CO_3, acetone, 0 °C, 2 h, 71%; [d] Levulinic acid, DIC, DMAP, DCM, 0 °C, 95%.

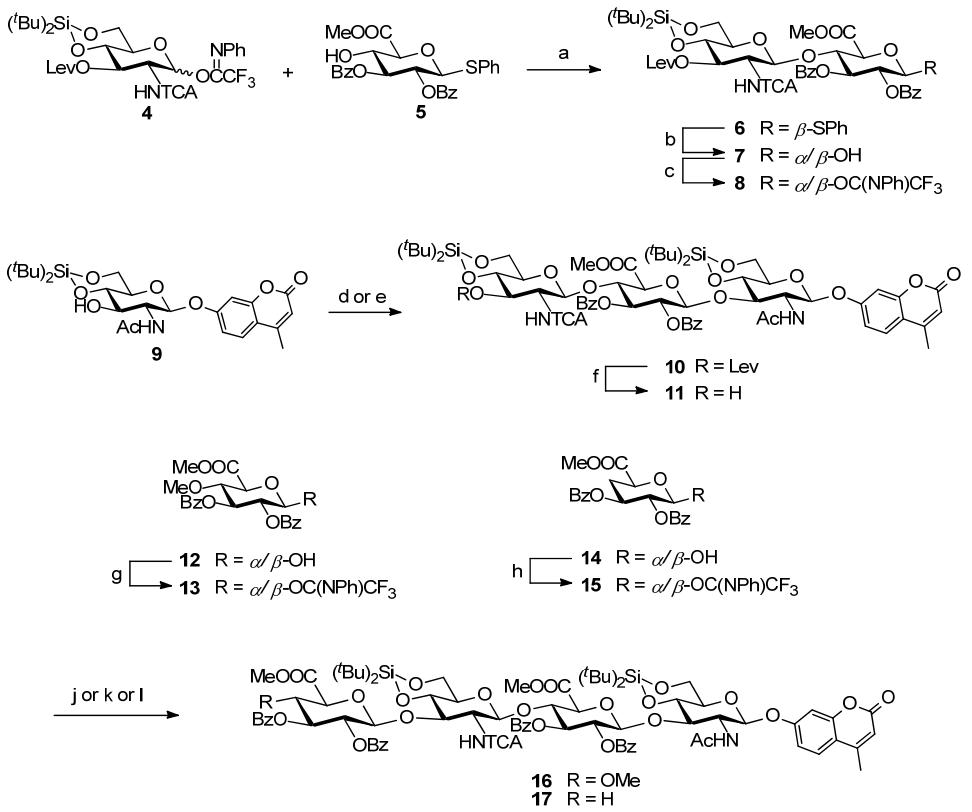
The synthesis of the tetrameric hyaluronidase probes **16** and **17** (Scheme 3) requires access to dimer **6**, which can be acquired by condensation of *N*-trichloroacetyl glucosamine donor **4** with glucuronic acid acceptor **5** (described in **Chapter 4**). The imidate donor **4** was synthesized (Scheme 2), starting from commercially available *α*-D-glucosamine hydrochloride, by the following transformations: Protection of the amine functionality by application of trichloroacetyl chloride in methanol/triethylamine produced *N*-trichloroacetyl-D-glucosamine **1**. The 4,6-di-*tert*-butylsilylene protective group was then introduced following the previously described procedure to give 4,6-protected hemiacetal **2**.[10] Regioselective introduction of the *N*-phenyl trifluoro-acetimidate to the anomeric position, according to the method of Valerio *et al.*,[14] produced the C3-OH D-glucosamine imidate **3**. The alcohol was then protected as a levulinic ester by

treatment with levulinic acid and diisopropylcarbodiimide, giving D-glucosamine donor **4** in 32% yield over 4 steps.

Having all monomeric building blocks available, the target tetrameric probes were assembled (Scheme 3). In the first glycosylation, *N*-phenyltrifluoroimidate donor **4** and methyl glucuronate **5** were condensed under the influence of triflic acid, to give hyaluronic acid dimer **6** in 97% yield.

As described in **Chapter 4**, the β-1,3-linkage between GlcA and GlcNAc-4MU can be formed by two different coupling strategies, namely the imidate and the dehydrative coupling methodologies. Therefore, hyaluronic acid dimer **6** was transformed into hemiacetal donor **7** by the NIS/TFA mediated hydrolysis[15] and subsequently into *N*-phenyl trifluoroacetimidate donor **8**.

Scheme 3. Synthesis of protected hyaluronic acid tetramers **16** and **17**.



Reagents and conditions: [a] TfOH, DCM, 0 °C, 4 h, 97%; [b] NIS, TFA, DCM, 0 °C, 2 h, 89%; [c] Cs_2CO_3, ClC(NPh)CF_3, acetone, 0 °C, 3 h, 85%. [d] **7**, Ph_2SO, Tf_2O, DCM −60 °C to −20 °C, 1 h, then **9**, −40 °C to 0 °C, 20 h, 33%; [e] **8** and **9**, TfOH, DCM, 0 °C, 2 h, 60%; [f] Hydrazine acetate, pyridine/acetic acid, r.t., 10 min., 99%. [g] Cs_2CO_3, ClC(NPh)CF_3, acetone, 0 °C, 3 h, 65%; [h] Cs_2CO_3, ClC(NPh)CF_3, acetone, 0 °C, 2 h, 73%; [j] **12**, Ph_2SO, Tf_2O, DCM −60 °C to −20 °C, 1 h, then **11**, −40 °C to 0 °C, 20 h, 46%; [k] **13** and **11**, TfOH, DCM, 0 °C, 4 h, 85%; [l] **15** and **11**, TfOH, DCM, 0 °C, 4 h, 78%.

Next, condensation of donors **7** and **8** with GlcNAc-4MU acceptor **9** was investigated. In contrast to the formation of the dimeric hyaluronidase substrates described in **Chapter 4**, a higher yield was obtained with the imidate procedure (60%) compared to the dehydrative condensation method (33%) in the formation of trimeric hyaluronic acid **10**. Deprotection of the C3′′-OLev protecting group with hydrazine in acetic acid gave hyaluronic acid trimer **11**.

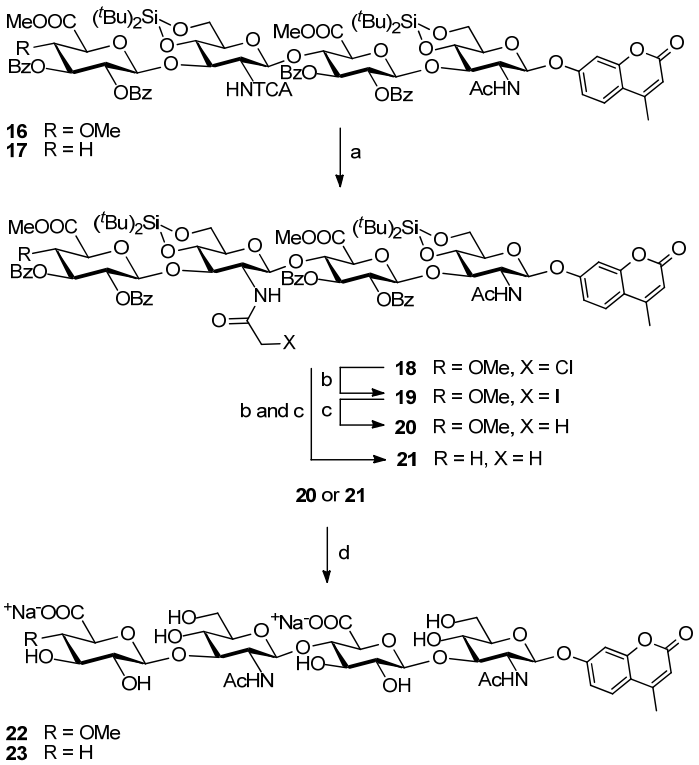
For the final glycosylation towards tetrameric hyaluronidase probes **16** and **17**, both the imidate and dehydrative condensation strategies were evaluated. The C4-methoxy imidate donor **13** was synthesized from the hemiacetal donor **12** and both donors were then utilized in the glycosylation of acceptor **11**. Also with these reaction partners, the imidate methodology proved to be superior in comparison with the dehydrative procedure, giving access to protected tetramer **16** in 85% yield. Following the same sequence of reactions, C4-deoxy hemiacetal **14** was first transformed into *N*-phenyl trifluoroimidate donor **15**, before being condensed with acceptor **11**, forming protected hyaluronic acid tetramer **17** in 78% yield (Scheme 3).

Several procedures were investigated for the deprotection of protected tetramers **16** and **17**, with special attention to the conversion of the *N*-TCA protective group into the *N*-acetyl functionality, in the presence of the coumarine lactone (Scheme 4).

Transformation of the *N*-TCA protective group to the corresponding *N*-acetyl functionality was evaluated with both tributylstannene/AIBN and zinc/acetic acid. The more frequently used radical approach proved unreliable with these substrates and a mixture of mono- and di-chloro acetyl products were observed.[16] The zinc/acetic acid mediated halide reduction also resulted in incomplete reduction of tetramer **16**.[17] This methodology produced the mono-chloro product **18** and the desired product **20** in a 4:1 ratio. Complete formation of product **20** from tetramer **16** could not be achieved, despite the fact that the reaction was subjected to extended reaction times with several additions of activated zinc. To overcome this problem the crude reaction mixture was subjected to the Finkelstein conditions, completely transforming the *N*-chloroacetyl derivative **18** into the *N*-iodoacetyl derivative **19**, which could be followed by HPLC–MS. The more easily reduced *N*-iodoacetyl derivate was then successfully transformed to the *N*-acetyl product **20** utilizing the zinc/acetic acid mediated reduction. Formation of the C4′′′′-deoxygenated tetramer **21** was similarly accomplished from the *N*-TCA protected tetramer **17** following the same reaction sequence as for **16** to **20** (Scheme 4).

The remaining protecting groups of the crude tetramers **20** and **21** were then removed following the sequence already developed for the dimeric hyaluronidase probes in **Chapter 4**: Sodium methoxide mediated removal of benzoyl protecting groups was followed by deprotection of the di-*tert*-butylsilylene protecting groups with hydrogen fluoride in pyridine. Finally, saponification of the remaining methylesters with aqueous sodium carbonate produced the crude tetrameric hyaluronidase probes. Purification by RP–HPLC (Scheme 4) produced tetramers **22** and **23** in 43% and 60% respectively, starting from **16** and **17**.

Scheme 4. Transformation of *N*-TCA to *N*-acetyl derivates **20** and **21** and final deprotection.



Reagents and conditions: [a] Zinc dust, AcOH, r.t., 40 h; [b] KI, acetone r.t., 20 h. [c] Zinc dust, AcOH, r.t., 20 h; [d] i) NaOMe cat., MeOH, DCM, 6 h; ii) $(HF)_3{\cdot}Et_3N$, pyridine, 18 h; iii) Na_2CO_3 aq., 4 h; iv) RP-HPLC, lyophilization and Amberlite-Na+ **22**: 43%; **23**: 60%.

With the tetrameric probes **22** and **23** in hand, their ability to probe mammalian hyaluronidase activity was investigated. Similar to the dimeric substrates described in **Chapter 4** were the tetrameric hyaluronic acid derivates processed by neither the bovine hyaluronidase (PH20) nor the human hyaluronidase 2 (Hyal2).

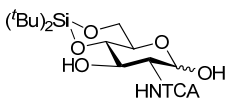
Conclusion

This chapter describes the synthesis of two tetrameric hyaluronidase probes. The probes were synthesized by condensation of *N*-trichloroacetyl-D-glucosamine-(*β*-1,4)-D-glucuronic acid dimer **6** and building blocks from **Chapter 4**. Zinc/acetic acid mediated reduction of the *N*-TCA protective group resulted in an *N*-chloroacetyl derivative that was converted into an *N*-iodoacetyl derivative *via* the Finkelstein reaction. The *N*-acetyl products **20** and **21** were acquired by zinc/acetic acid reduction of the intermediate *N*-iodoacetyl derivative. Global deprotection and purification by RP–HPLC produced the final products in good yields. However, the tetrameric hyaluronidase probes were not applicable for the monitoring of mammalian hyaluronidase activity.

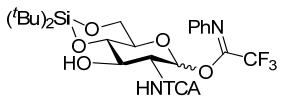
Experimental section

General Procedures and Material: Commercially available reagents and solvents (Acros, Fluka or Merck) were used as received unless stated otherwise. DCM and THF were freshly distilled, before use, over P_2O_5 and Na/benzophenone respectively. Triethylamine was destilled over calcium hydride and stored over KOH. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. All moisture sensitive reactions were performed under an argon atmosphere. Traces of water was removed from starting compounds by coevaporation with DCE, dioxane and/or toluene. Molecular sieves 3Å were flamedried prior to use. Liquid column chromatography was performed using forced flow of the indicated solvent systems on Screening Devices Silica gel 60 (40–63 μm mesh). Size exclusion chromatography was performed on Sephadex LH20 (eluent MeOH/DCM, 1:1). Analytical TLC was performed on aluminium sheets, pre-coated with silica gel (Merck, silica gel 60, F_{254}). Compounds were visualized with UV absorption (245 nm), by spraying with either 20% H_2SO_4 in ethanol, or ammonium molybdate/cerium sulphate solution [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_6\cdot 2H_2O$ (10 g/L), 10% sulphuric acid in ethanol], or phosphormolybdic acid in EtOH (150 g/L) followed by charring (~150 °C) or by spraying with an aqueous solution of potassium permanganate [$KMnO_4$ (20 g/L), K_2CO_3 (10 g/L)]. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter (Sodium D-line, λ = 589 nm). ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 (^1H) and 100.6 (^{13}C) MHz or on a Bruker AV 500 MHz spectrometer at 500.0 (^1H) and 125.1 (^{13}C) MHz respectively. Chemical shifts are reported as δ values (ppm) and directly referenced to TMS (0.00 ppm) in $CDCl_3$ or *via* the solvent residual peak (D_2O). Coupling constants (*J*) are given in Hz and all ^{13}C spectra are proton decoupled. NMR assignments were made using COSY and HSQC and in some cases TOCSY experiments. HPLC-MS analyses were performed on a LCQ Advantage Max (Thermo Finnigan) equipped with a Gemini C_{18} column (Phenomenex, 50 x 4.6 mm, 3μ), utilizing the following buffers: A: H_2O, B: acetonitrile and C: 1.0% $TFA_{(aq)}$. HPLC purifications were performed on a Gilson GX-281 automated HPLC system, equipped with a preparative Gemini C_{18} column (Phenomenex, 150 x 21.20, 5 μ). Products were eluted using the following buffers: A: ammonium acetate (20 $mM_{(aq)}$) or triethylammonium acetate (50 $mM_{(aq)}$), B: acetonitrile (HPLC-grade), 20 mL/min. Purified products were lyophilised on a CHRIST ALPHA 2–4 LD_{PLUS} to remove water and traces of buffer salts. Hyaluronidase enzyme was purchased from Sigma Aldrich (3506), Type I-S, originating from bovine testes, 290 units/mg solid. The assay were performed using Mac Ilvain buffer at pH 4.0, 5.0 and 6.0 and three different substrate concentrations: 0.1, 0.5 and 1.0 mM. 20–50 μg hyaluronidase or 25 μL waterextract from spleen control. The assays were performed at 37 °C, during 2 hours and was then quenched by adding 0.3 M glycine-NaOH (pH 10.6). The fluorescence were measured at 366 nm (exc.) and 445 nm (em.).

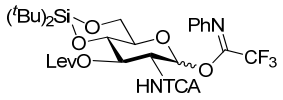
2-Deoxy-2-trichloroacetamido-*α*/*β*-D-glucopyranoside (1) To a solution of D-glucosamine·HCl (37.8 g, 175 mmol, 1.0 eq) in and methanol (300 mL) were triethylamine (72.8 mL, 525 mmol, 3.0 eq) added, followed by dropwise addition of trichloroacetylchloride (21.6 mL, 193 mmol, 1.1 eq) at 0 °C. The reaction was allowed to reach room temperature. After 3 days was the solids removed by suction filtration and the mothe liquor was concentrated *in vacuo*. Purification by column chromatography (0–5% MeOH in EtOAc) yielded the title compound as a white solid in a 5:1 *α*/*β*-ratio (28.8 g, 88 mmol, 51%). R_f = 0.60 (*α*) and 0.39 (*β*) (20% MeOH in EtOAc); Analytical data are identical to literature precedence.[11]



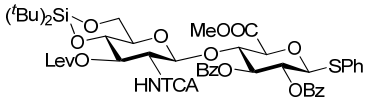
4,6-*O*-Di-*tert*-butylsilylanediyl-2-deoxy-2-trichloroacetamido-*α*/*β*-D-glucopyranoside (2) To a solution of **1** (9.3 g, 29 mmol, 1.0 eq) in DMF (300 mL), di-*tert*-butylsilanediyl bistriflate (8.8 mL, 27 mmol, 0.95 eq) was added dropwise at −40 °C. The reaction was stirred for one hour and pyridine (6.9 mL, 82 mmol, 3.0 eq) was added and the reaction was subsequently diluted with diethylether (500 mL) and washed with water (2x 500 mL). The organics were dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (50% EtOAc in petroleum ether) yielded the title compound as a white solid (11.7 g, 25.2 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.98 (d, 1 H, *J* = 8.4 Hz, NH), 5.34 (d, 1 H, *J* = 3.2 Hz, H-1), 4.13 – 3.96 (m, 3 H, H-2, H-6_a and H-3), 3.94 - 3.87 (m, 2 H, H-4 and H-5), 3.77 (m, 1 H, H-6_b), 3.30 (bs, 1 H, OH), 1.06 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.99 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.3 ($C{=}O_{TCA}$), 91.7 (C-1), 77.7 (C-4), 71.9 (C-3), 66.4 (C-5), 66.3 (C-6), 54.4 (C-2), 27.4 ($CH_{3\text{-}tBu\text{-}Si}$), 26.9 ($CH_{3\text{-}tBu\text{-}Si}$), 22.7 ($C_{q\text{-}tBu\text{-}Si}$), 19.7 ($C_{q\text{-}tBu\text{-}Si}$); IR (neat): 2930, 2337, 1681, 1533, 1092, 1064, 817, 765 cm^{-1}; HRMS Calcd for $[C_{16}H_{28}Cl_3NO_6Si + H]^+$: 464.0824, found 464.0823.



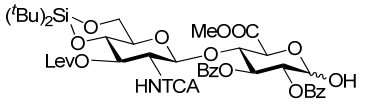
4,6-*O*-Di-*tert*-butylsilanediyl-2-deoxy-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)-2-trichloroacetamido-*α*/*β*-D-glucopyranoside (3) To a solution of **2** (0.50 g, 1.1 mmol, 1.0 eq) in acetone (4 mL) were cesium carbonate (0.40 g, 1.3 mmol, 1.2 eq) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (0.33 mL, 1.6 mmol, 1.5 eq) added at 0 °C. After full conversion to product as measured by TLC was the mixture filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–5% acetone in petroleum ether) yielded the title imidate **3** as a colourless oil (0.48 g, 0.76 mmol, 71%). R_f = 0.32 (*α*) and 0.19 (*β*) (10% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34 – 7.27 (m, 2 H, H_{arom}), 7.11 (t, 1 H, *J* = 7.2 Hz, H_{arom}), 6.84 (d, 1 H, *J* = 7.2 Hz, NH), 6.78 (d, 2 H, *J* = 7.6 Hz, H_{arom}), 6.40 (bs, 1 H, H-1), 4.20 – 4.16 (m, 2 H, H-2, H-6_a), 3.98 (t, 1 H, *J* = 8.8 Hz, H-3), 3.96 – 3.84 (m, 3 H, H-4, H-5 and H-6_b), 2.84 (bs, 1 H, OH), 1.07 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 1.01 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.2 ($C{=}O_{TCA}$), 142.8 ($C_{q\text{-}arom}$), 128.8, 124.7, 119.2 (CH_{arom} x3), 93.0 (C-1), 92.0 ($C_{q\text{-}CCl3}$), 77.0 (C-4), 71.3 (C-3), 68.5 (C-5), 66.0 (C-6), 54.3 (C-2), 27.3, 26.8 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 22.7, 19.9 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 2931, 2337, 1683, 1533, 1092, 1064, 817, 765 cm^{-1}; HRMS Calcd for $[C_{24}H_{32}Cl_3F_3N_2O_6Si + H]^+$: 635.1120, found 635.1120.



4,6-*O*-Di-*tert*-butylsilanediyl-2-deoxy-4-*O*-levulinoyl-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)- 2-trichloroacetamido-*α*/*β*-D-glucopyranoside (4) Trifluoroacetimidate **3** (4.2 g, 6.3 mmol, 1.0 eq) and levulinic acid (2.1 g, 18 mmol, 2.8 eq) were dissolved in anhydrous DCM (40 mL) at 0 °C. *N*,*N*'-diisopropylcarbodiimide (1.4 mL, 8.9 mmol, 1.4 eq) was added followed by a catalytic amount of DMAP (38 mg, 0.32 mmol, 0.05 eq). The reaction was stirred for 4 hours at 0 °C and then at room temperature over night. The reaction mixture was then filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–5% acetone in petroleum ether) gave the title compound as a colourless oil (4,4 g, 6.0 mmol, 95%). R_f *α*/*β* = 0.63 (30% acetone in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27 (t, 2 H, *J* = 7.6 Hz, H_{arom}), 7.11 (t, 1 H, *J* =7.6 Hz, H_{arom}), 6.84 (d, 1 H, *J* = 7.6 Hz, NH), 6.78 (d, 2 H, *J* = 8.0 Hz, H_{arom}), 6.43 (bs, 1 H, H-1), 5.27 (t, 1 H, *J* = 10.0 Hz, H-3), 4.27 (m, 1 H, H-2), 4.17 (m, 1 H, H-6_a), 3.98 (t, 1 H, *J* = 9.2 Hz, H-4), 3.97 – 3.88 (m, 2 H, H-5, H-6_b), 2.75 – 2.70 (m, 2 H, $CH_{2\text{-}Lev}$), 2.65 – 2.62 (m, 2 H, $CH_{2\text{-}Lev}$), 2.13 (s, 3 H, $CH_{3\text{-}Lev}$), 1.07 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.99 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 205.2 ($C{=}O_{Lev\text{-}ketone}$), 173.5 ($C{=}O_{Lev\text{-}ester}$), 162.0 ($C{=}O_{TCA}$), 142.5 ($C_{q\text{-}arom}$), 129.0, 124.6, 119.4 ($CH_{arom}$ x3), 92.6 (C-1), 91.6 ($C_{q\text{-}CCl3}$), 73.8 (C-4), 71.8 (C-3), 68.8 (C-5), 65.9 (C-6), 53.5 (C-2), 37.7 ($CH_{2\text{-}Lev}$), 29.4 ($CH_{3\text{-}Lev}$), 27.7 ($CH_{2\text{-}Lev}$), 27.1, 26.6 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 22.4, 19.7 ($C_{q\text{-}tBu\text{-}Si}$ x2); HRMS Calcd for $[C_{29}H_{38}Cl_3F_3N_2O_8Si + Na]^+$: 755.1307, found 755.1310.

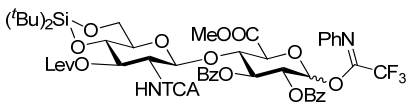


Methyl (phenyl 2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-1-thio-*β*-D-glucopyranoside) uronate (6) A mixture of glucuronic acid acceptor **5** (4.0 g, 7.8 mmol, 1.0 eq) and imidate donor **4** (6.9 g, 9.4 mmol, 1.2 eq) was coevaporated with anhydrous toluene two times, dissolved in anhydrous DCM (10 mL) and stirred with activated molsieves (3Å) for one hour. Triflic acid (40 µL, 0.45 mmol, 0.06 eq) was added at 0 °C and the reaction mixture was stirred for 4 hours. Anhydrous triethylamine (0.2 mL) was added to quench the reaction. The mixture was transferred to an extraction funnel using EtOAc (40 mL) and was then washed with sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were then dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by size exclusion chromatography followed by column chromatography (0–20% acetone in petroleum ether) yielded the title disaccharide as a white solid (7.9 g, 7.6 mmol, 97%). R_f = 0.24 (50% EtOAc in petroleum ether); $[\alpha]_D^{22}$: -16 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 – 7.92 (m, 4 H, H_{arom}), 7.58 – 7.51 (m, 2 H, H_{arom}), 7.50 – 7.46 (m, 2 H, H_{arom}), 7.44 – 7.38 (m, 4 H , H_{arom}), 7.35 – 7.31 (m, 3 H, H_{arom}), 6.79 (d, 1 H, *J* = 9.0 Hz, NH), 5.66 (t, 1 H, *J* = 9.2 Hz, H-3), 5.40 (t, 1 H, *J* = 9.7 Hz, H-2), 5.02 (dd, 1 H, *J* = 10.7, 9.0 Hz, H-3'), 4.99 (d, 1 H, *J* = 9.9 Hz, H-1), 4.93 (d, 1 H, *J* = 8.3 Hz, H-1'), 4.25 (t, 1 H, *J* = 9.3 Hz, H-4), 4.14 (d, 1 H, *J* = 9.7 Hz, H-5), 3.89 (s, 3 H, $CH_{3\text{-}COOMe}$), 3.86 (m, 1 H, H-2'), 3.57 (t, 1 H, *J* = 9.3 Hz, H-4'), 3.47 (dd, 1 H, *J* = 10.4, 4.9 Hz, H-6_a'), 3.26 (ddd, 1 H, *J* = 9.8, 9.3, 4.9 Hz, H-5'), 2.73 – 2.69 (m, 2 H, $CH_{2\text{-}Lev}$), 2.61 – 2.55 (m, 3 H, H-6_b', $CH_{2\text{-}Lev}$), 2.16 (s, 3 H, $CH_{3\text{-}Lev}$), 0.90 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.89 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 205.7 ($C{=}O_{Lev\text{-}ketone}$), 172.4 ($C{=}O_{Lev\text{-}ester}$), 168.6 ($C{=}O_{COOMe}$), 165.1, 165.0 ($C{=}O_{Bz}$ x2), 161.6 ($C{=}O_{TCA}$), 133.4, 133.1, 132.8 (CH_{arom} x3), 131.7, 129.9 ($C_{q\text{-}arom}$ x2), 129.8, 129.7 (CH_{arom} x2), 129.0 ($C_{q\text{-}arom}$), 128.9, 128.42, 128.39, 128.37 (CH_{arom} x4), 100.5 (C-1'), 92.3 ($C_{q\text{-}CCl3}$), 86.9 (C-1), 77.0 (C-5), 76.4 (C-4), 74.5 (C-4'), 74.4 (C-3'), 73.7 (C-3), 70.6 (C-5'), 69.7 (C-2), 64.9 (C-6'), 55.7 (C-2'), 53.3 ($CH_{3\text{-}COOMe}$), 38.0 ($CH_{2\text{-}Lev}$), 29.6 ($CH_{3\text{-}Lev}$), 28.0 ($CH_{2\text{-}Lev}$), 27.3, 26.7 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 22.4, 19.7 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 2936, 2861, 1736, 1522, 1272, 1070, 1026, 828, 710 cm^{-1}; HRMS Calcd for $[C_{48}H_{56}Cl_3NO_{15}SSi + Na]^+$: 1074.2098, found 1074.2105.

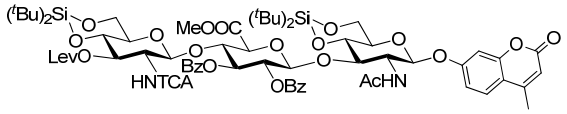


Methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-*α*/*β*-D-glucopyranose) uronate (7) To a solution of disaccharide **6** (4.5 g, 4.3 mmol, 1.0 eq) in DCM (150 mL) were *N*-iodosuccinimide (1.9 g, 8.6 mmol, 2.0 eq) and trifluoroacetic acid (0.32 mL, 4.3 mmol, 1.0 eq) added at 0 °C. After 2 hours was the mixture quenched by addition of triethylamine (1 mL) and transferred to an extraction funnel using DCM (100 mL). The organics were washed with sat. aq. $NaHCO_3$ (300 mL) and brine (300 mL). The aqueous layers were then extracted with EtOAc (300 mL) and the combined organics were dried ($MgSO_4$), filtrated and concentrated *in vacuo*. Purification by column chromatography (10–40% EtOAc in petroleum ether) yielded the disaccharide hemiacetal **7** as a white solid in a 4:1 *α*/*β*-ratio (3.7 g, 3.8 mmol, 89%). R_f = 0.19 (50% EtOAc in petroleum ether); NMR assignment for the major isomer (*α*): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 – 7.93 (m, 4 H, H_{arom}), 7.56 – 7.45 (m, 2 H, H_{arom}), 7.43 – 7.37 (m, 2 H, H_{arom}), 7.35 – 7.30 (m, 2 H, H_{arom}), 6.85 (d, 1 H, *J* = 8.9 Hz, NH), 5.97 (dd, 1 H, *J* = 10.0, 8.9 Hz, H-3), 5.68 (d, 1 H, *J* = 3.4 Hz, H-1), 5.21 (dd, 1 H, *J* = 10.0, 3.4 Hz, H-2), 5.02 (dd, 1 H, *J* = 10.7, 9.1 Hz, H-3'), 4.97 (d, 1 H, *J* = 8.3 Hz, H-1'), 4.61 (d, 1 H, *J* = 9.5 Hz, H-5), 4.21 (t, 1 H, *J* = 9.2 Hz, H-4), 3.86 – 3.81 (m, 4 H, H-2' and $CH_{3\text{-}COOMe}$), 3.61 (bs, 1 H, OH), 3.56 (t, 1 H, *J* = 9.2 Hz, H-4'), 3.49 (dd, 1 H, *J* = 10.4, 5.0 Hz, H-6_a'), 3.25 (ddd, 1 H, *J* = 9.9, 9.8, 5.0 Hz, H-5'), 2.71 – 2.66 (m, 3 H, H-6_b' and $CH_{2\text{-}Lev}$), 2.57 – 2.54 (m, 2 H, $CH_{2\text{-}Lev}$), 2.04 (s, 3 H, $CH_{3\text{-}Lev}$), 0.88 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.87 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 205.9 ($C{=}O_{Lev\text{-}ketone}$), 172.4 ($C{=}O_{Lev\text{-}ester}$), 170.1 ($C{=}O_{COOMe}$), 165.8,

165.1 ($C{=}O_{Bz}$ x2), 161.7 ($C{=}O_{TCA}$), 133.4, 133.1 (CH_{arom} x2), 130.1 ($C_{q\text{-}arom}$), 129.9, 129.6 (CH_{arom} x2), 128.9 ($C_{q\text{-}arom}$), 128.5, 128.4 (CH_{arom} x2), 100.5 (C-1′), 92.3 ($C_{q\text{-}CCl3}$), 90.5 (C-1), 76.7 (C-4), 74.4 (C-3′), 74.2 (C-4′), 71.0 (C-5′), 70.6 (C-2), 69.7 (C-3), 69.5 (C-5), 65.0 (C-6′), 55.8 (C-2′), 53.1 ($CH_{3\text{-}COOMe}$), 38.8 (CH_2-Lev), 29.7 (CH_3-Lev), 27.8 (CH_2-Lev), 27.2, 26.7 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 22.4, 19.7 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 3362, 2935, 2860, 1722, 1526, 1276, 1070, 827, 710 cm^{-1}; HRMS Calcd for $[C_{42}H_{52}Cl_3NO_{16}Si + Na]^+$: 982.2013, found 928.2018.

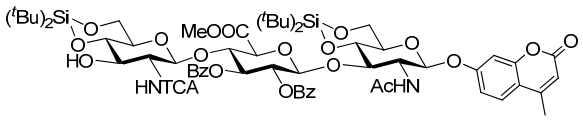


Methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)-*α/β*-D-glucopyranoside) uronate (8) To a solution of disaccharide hemiacetal **7** (3.7 g, 3.8 mmol, 1.0 eq) in acetone (10 mL) were cesium carbonate (1.9 g, 5.8 mmol, 1.5 eq) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (0.88 mL, 5.8 mmol, 1.5 eq) added at 0 °C. After 1.5 hours was the mixture filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–15% acetone in petroleum ether) produced the title disaccharide imidate as a colorless oil in a 1:3 *α/β*-ratio (3.712 g, 3.28 mmol, 85%). R_f = 0.22 (50% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.01 – 7.93 (m, 4 H, H_{arom}), 7.56 – 7.50 (m, 2 H, H_{arom}), 7.44 – 7.35 (m, 4 H, H_{arom}), 7.27 (t, 0.3 H, *J* = 7.8 Hz, $H_{arom\text{-}NPh\text{-}\alpha}$), 7.16 – 7.07 (m, 2 H, H-1, $H_{arom\text{-}NPh\text{-}\alpha/\beta}$), 7.01 (t, 0.7 H, *J* = 7.4 Hz, $H_{arom\text{-}NPh\text{-}\beta}$), 6.93 (d, 0.3 H, *J* = 8.9 Hz, NH_α), 6.92 (d, 0.7 H, *J* = 8.7 Hz, NH_β), 6.79 (bd, 0.7 H, *J* = 8.0 Hz, H-1_β), 6.77 (bs, 0.6 H, $H_{arom\text{-}NPh\text{-}\alpha}$), 6.43 (bd, 1.4 H, *J* = 6.2 Hz, $H_{arom\text{-}NPh\text{-}\beta}$), 6.23 (bs, 0.3 H, H-$1_\alpha$), 5.95 (t, 0.7 H, *J* = 9.6 Hz, H-3_β), 5.74 (t, 0.3 H, *J* = 7.6 Hz, H-3_α), 5.56 (m, 1 H, H-2_β and H-2_α), 5.11 (dd, 0.7 H, *J* = 10.7, 9.0 Hz, H-3_β′), 5.06 (dd, 0.7 H, *J* = 10.5, 8.8 Hz, H-3_α′), 5.03 (d, 0.7 H, *J* = 8.2 Hz, H-1_β′), 4.98 (d, 0.3 H, *J* = 8.2 Hz, H-1_α′), 4.54 – 4.48 (m, 1 H, H-5_α and H-5_β), 4.44 (t, 0.3 H, *J* = 7.9 Hz, H-4_α), 4.38 (t, 0.7 H, *J* = 9.4 Hz, H-4_β), 3.87 (s, 2.1 H, $CH_{3COOMe\text{-}\beta}$), 3.86 – 3.79 (m, 1 H, H-2_α′ and H-2_β′), 3.78 (s, 0.9 H, $CH_{3COOMe\text{-}\alpha}$), 3.64 – 3.55 (m, 1.7 H, H-4_β′, H-$6_{a\text{-}\alpha}$′ and H-$6_{a\text{-}\beta}$′), 3.51 (t, 0.3 H, *J* = 9.3 Hz, H-4_α′), 3.31 (td, 0.3 H, *J* = 9.7, 4.8 Hz, H-5_α′), 3.27 (td, 0.7 H, *J* = 9.9, 4.9 Hz, H-5_β′), 2.83 – 2.66 (m, 3 H, H-$6_{b\text{-}\alpha}$′, H-$6_{b\text{-}\beta}$′ and $CH_{2\text{-}Lev\text{-}\alpha/\beta}$), 2.60 – 2.54 (m, 2 H, $CH_{2\text{-}Lev\text{-}\alpha/\beta}$), 2.13 (s, 2.1 H, $CH_{3\text{-}Lev\text{-}\beta}$), 2.12 (s, 0.9 H, $CH_{3\text{-}Lev\text{-}\alpha}$), 0.91 – 0.89 (m, 9 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$), 0.88 (s, 2.7 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 0.87 (s, 6.3 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 205.7, 205.6 ($C{=}O_{Lev\text{-}ketone\text{-}\alpha/\beta}$), 172.2 ($C{=}O_{Lev\text{-}ester\text{-}\alpha}$), 172.0 ($C{=}O_{Lev\text{-}ester\text{-}\beta}$), 168.7 ($C{=}O_{COOMe\text{-}\alpha}$), 168.5 ($C{=}O_{COOMe\text{-}\beta}$), 165.1 ($C{=}O_{Bz\text{-}\beta}$), 164.9 ($C{=}O_{Bz\text{-}\alpha}$), 164.8 ($C{=}O_{Bz\text{-}\beta}$), 164.7 ($C{=}O_{Bz\text{-}\alpha}$), 161.6 ($C{=}O_{TCA\text{-}\beta}$), 161.5 ($C{=}O_{TCA\text{-}\alpha}$), 142.7 ($C_{q\text{-}arom\text{-}NPh\text{-}\alpha}$), 142.5 ($C_{q\text{-}arom\text{-}NPh\text{-}\beta}$), 133.6, 133.4, 133.2, 133.1, 129.7 ($CH_{arom\text{-}\alpha/\beta}$ x5), 129.61 ($C_{q\text{-}arom\text{-}\alpha/\beta}$), 129.59 , 129.5 ($CH_{arom\text{-}\alpha/\beta}$ x2), 129.4 ($C_{q\text{-}arom\text{-}\alpha/\beta}$), 128.6, 128.5, 128.4, 128.3, 128.2 ($CH_{arom\text{-}\alpha/\beta}$ x5), 128.0 ($C_{q\text{-}arom\text{-}\alpha/\beta}$), 124.5, 124.3, 119.1, 118.8 ($CH_{arom\text{-}NPh\text{-}\alpha/\beta}$ x4), 100.8 (C-1_α′), 100.0 (C-1_β′), 94.4 (C-1_α), 92.6 ($C_{q\text{-}CCl3\text{-}\alpha}$), 92.3 ($C_{q\text{-}CCl3\text{-}\beta}$), 91.7 (C-$1_\beta$), 75.8 (C-$4_\beta$), 75.6 (C-$4_\alpha$), 74.4 (C-$4_\beta$′), 74.3 (C-$4_\alpha$′), 74.2 (C-$3_\alpha$′), 73.8 (C-$3_\beta$′), 71.6 (C-$5_{\alpha/\beta}$), 70.5 (C-$5_{\alpha/\beta}$′), 70.4 (C-$3_\alpha$), 70.2 (C-$2_\alpha$), 69.6 (C-$3_\beta$), 69.3 (C-$2_\beta$), 64.9 (C-$6_{\alpha/\beta}$′), 55.9 (C-$2_\beta$′), 55.7 (C-$2_\alpha$′), 53.3 ($CH_{3\text{-}COOMe\text{-}\beta}$), 53.0($CH_{3\text{-}COOMe\text{-}\beta}$), 37.9 ($CH_{2\text{-}Lev\text{-}\alpha/\beta}$), 29.5 ($CH_{3\text{-}Lev\text{-}\alpha/\beta}$), 29.2, 27.9 ($CH_{2\text{-}Lev\text{-}\alpha/\beta}$), 27.1, 26.6 ($CH_{3\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$ x2), 22.3, 19.6 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$ x2); IR (neat): 2935, 2861, 2360, 1718, 1526, 1268, 1070, 907, 827, 729 cm^{-1}.



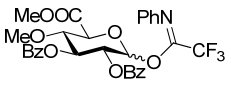
4-Methylumbelliferyl 2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-levulinoyl-2-trichloro-acetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl uronate)-*β*-D-glucopyranoside (10) Method A: A mixture of imidate donor **8** (3.0 g, 2.7 mmol, 1.2 eq) and *N*-acetyl glucosamine acceptor **9** (1.2 g, 2.2 mmol, 1.0 eq) was coevaporated two

times with anhydrous toluene, dissolved in anhydrous DCM (10 mL) and stirred over activated molsieves (3Å) for one hour. Triflic acid (20 µL, 0.23 mmol, 0.1 eq) was added at 0 °C and the reaction mixture was stirred overnight at 4 °C. Anhydrous triethylamine (0.2 mL) was added and the reaction was transferred to an extraction funnel using EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were dried ($MgSO_4$), filtrated and concentrated *in vacuo*. Purification by size exclusion chromatography followed by column chromatography (20–40% EtOAc in petroleum ether) yielded trisaccharide **10** as a white solid (2.0 g, 1.3 mmol, 60%). **Method B:** A mixture of 1-hydroxy disaccharide donor **7** (0.12 g, 0.12 mmol, 1.2 eq) and diphenyl sulfoxide (57 mg, 0.28 mmol, 2.8 eq) was coevaporated with anhydrous toluene (x2) and was then dissolved in anhydrous DCM (3 mL) and stirred over activated molsieves (3Å) for one hour. Tf_2O (24 µL, 1.4 mmol, 1.4 eq) was added at −60 °C and the mixture was stirred for one hour at −20 °C. A solution of the *N*-acetyl glucosamine acceptor **9** (52 mg, 0.10 mmol, 1.0 eq), coevaporated with anhydrous toluene, was dissolved in anhydrous DCM (1 mL) and added to the activated donor at −40 °C. The reaction was stirred for 2 hours going to 0 °C. The reaction was quenched with anhydrous triethylamine (0.2 mL) and was then diluted with DCM (20 mL), washed with sat. aq. $NaHCO_3$ (20 mL) and brine (20 mL). The aqueous layers were extracted with EtOAc (30 mL) and the combined organics were dried ($MgSO_4$), filtrated and concentrated *in vacuo*. Purification by size exclusion chromatography followed by column chromatography (20–40% EtOAc in petroleum ether) yielded trisaccharide **10** as a white solid (48 mg, 0.033 mmol, 33%). $[\alpha]_D^{22}$: −5 (C = 0.43, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 - 7.92 (m, 4 H, H_{arom}), 7.57 - 7.52 (m, 2 H, H_{arom}), 7.45 (d, 1 H, *J* = 9.4 Hz, H-5$_{4\text{-MU}}$), 7.43 - 7.38 (m, 4 H, H_{arom}), 6.89 (d, 1 H, *J* = 8.8 Hz, NH_{TCA}), 6.86 - 6.82 (m, 2 H, H-6$_{4\text{-MU}}$ and H-8$_{4\text{-MU}}$), 6.14 (d, 1 H, *J* = 1.1 Hz, H-3$_{4\text{-MU}}$), 5.75 (d, 1 H, *J* = 7.5 Hz, NH_{NAc}), 5.65 (d, 1 H, *J* = 8.2 Hz, H-1), 5.59 (t, 1 H, *J* = 9.1 Hz, H-3′), 5.36 (dd, 1 H, *J* = 9.1, 6.5 Hz, H-2′), 5.19 (d, 1 H, *J* = 6.5 Hz, H-1′), 5.02 (dd, 1 H, *J* = 10.6, 9.2 Hz, H-3′′), 4.89 (d, 1 H, *J* = 8.3 Hz, H-1′′), 4.38 (dd, 1 H, *J* = 10.1, 8.4 Hz, H-3), 4.33 (t, 1 H, *J* = 9.2 Hz, H-4′), 4.19 (dd, 1 H, *J* = 10.3, 5.0 Hz, H-6$_a$), 4.11 (d, 1 H, *J* = 9.2 Hz, H-5′), 3.96 (dd, 1 H, *J* = 9.5, 8.4 Hz, H-4), 3.88 (dd, 1 H, *J* = 10.3, 10.0, H-6$_b$), 3.84 – 3.79 (m, 4 H, $CH_{3\text{-COOMe}}$ and H-2′′), 3.67 - 3.50 (m, 3 H, H-5, H-6a′′ and H-4′′), 3.44 (ddd, 1 H, *J* = 10.1, 8.2, 7.5 Hz, H-2), 3.26 (td, 1 H, *J* = 9.9, 4.9 Hz, H-5′′), 2.72 - 2.65 (m, 3 H, $CH_{2\text{-Lev}}$ and H-6b′′), 2.56 (t, 2 H, *J* = 6.8 Hz, $CH_{2\text{-Lev}}$), 2.37 (d, 3 H, *J* = 1.1 Hz, $CH_{3\text{-4-MU}}$), 2.13 (s, 3 H, $CH_{3\text{-Lev}}$), 1.79 (s, 3 H, $CH_{3\text{-NAc}}$), 1.05 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 0.98 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 0.89 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 0.87 (s, 9 H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 205.8 ($C{=}O_{\text{Lev-ketone}}$), 172.3 ($C{=}O_{\text{Lev-ester}}$), 170.7 ($C{=}O_{NAc}$), 169.3 ($C{=}O_{COOMe}$), 165.3, 165.1 ($C{=}O_{Bz}$ x2), 161.7 (C-2$_{4\text{-MU}}$), 160.9 ($C{=}O_{TCA}$), 159.5, 154.7, 152.3 (C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 133.7, 133.2 (CH_{arom} x2), 129.9 ($C_{q\text{-arom}}$), 129.7, 129.6 (CH_{arom} x2), 129.0 ($C_{q\text{-arom}}$), 128.6, 128.4 (CH_{arom} x2), 125.6 (C-5$_{4\text{-MU}}$), 115.2 (C-4$_{4\text{-MU}}$), 113.5 (C-6$_{4\text{-MU}}$), 112.9 (C-3$_{4\text{-MU}}$), 104.1 (C-8$_{4\text{-MU}}$), 100.5 (C-1′), 100.4 (C-1′′), 97.2 (C-1), 92.4 ($C_{q\text{-CCl3}}$), 79.2 (C-3), 76.4 (C-4), 76.2 (C-4′), 74.4 (C-4′′), 74.3 (C-3′′), 74.0 (C-5′), 72.8 (C-2′), 72.6 (C-3′), 70.6 (C-5′′), 70.4 (C-5), 66.1 (C-6), 65.0 (C-6′′), 57.1 (C-2), 55.8 (C-2′′), 53.1 ($CH_{3\text{-COOMe}}$), 38.0 ($CH_{2\text{-Lev}}$), 29.8 ($CH_{3\text{-Lev}}$), 28.0 ($CH_{2\text{-Lev}}$), 27.3, 27.2, 26.9, 26.7 ($CH_{3\text{-tBu-Si}}$ x4), 23.3 ($CH_{3\text{-NAc}}$), 22.6, 22.4, 19.9, 19.7 ($C_{q\text{-tBu-Si}}$ x4), 18.6 ($CH_{3\text{-4-MU}}$); IR (neat): 2934, 2860, 1718, 1270, 1069, 827, 709 cm^{-1}; HRMS Calcd for $[C_{68}H_{87}Cl_3N_2O_{32}Si_2 + H]^+$: 1461.4377, found 1461.4393.

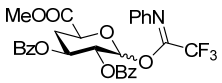


4-Methylumbelliferyl 2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl uronate)-*β*-D-glucopyranoside (11) To a solution of trisaccharide **10** (1.9 g, 1.3 mmol, 1.0 eq) in pyridine:acetic acid 4:1 (60 mL), was hydrazine monohydrate (0.32 g, 6.5 mmol, 5.0 eq) added

at room temperature. At full conversion of starting material (TLC), was the mixture diluted with EtOAc (100 mL) and washed with sat. aq. $NaHCO_3$ (200 mL) and brine (100 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried ($MgSO_4$), filtrated and concentrated *in vacuo*. Purification by column chromatography (20–50% EtOAc in petroleum ether) yielded the title compound as a white solid (1.8 g, 1.3 mmol, 99%).$[\alpha]_D^{22}$: +7 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 - 7.91 (m, 4 H, H_{arom}), 7.56 - 7.50 (m, 2 H, H_{arom}), 7.45 (d, 1 H, *J* = 9.6 Hz, H-5$_{4\text{-MU}}$), 7.42 - 7.37 (m, 4 H, H_{arom}), 7.06 (d, 1 H, *J* = 7.5 Hz, NH_{TCA}), 6.85 (dd, 1 H, *J* = 9.6, 2.5 Hz, H-6$_{4\text{-MU}}$), 6.84 (d, 1 H, *J* =2.5 Hz, H-8$_{4\text{-MU}}$), 6.13 (d, 1 H, *J* = 1.1 Hz, H-3$_{4\text{-MU}}$), 5.82 (d, 1 H, *J* = 7.5 Hz, NH_{NAc}), 5.64 (d, 1 H, *J* = 8.2 Hz, H-1), 5.58 (t, 1 H, *J* = 9.1 Hz, H-3′), 5.38 (dd, 1 H, *J* = 9.2, 6.6 Hz, H-2′), 5.21 (d, 1 H, *J* = 6.5 Hz, H-1′), 4.94 (d, 1 H, *J* = 8.3 Hz, H-1′′), 4.41 – 4.32 (m, 2 H, H-3 and H-4′), 4.19 (dd, 1 H, *J* = 10.3, 5.1 Hz, H-6$_a$), 4.15 (d, 1 H, *J* = 9.3 Hz, H-5′), 3.97 (dd, 1 H, *J* = 9.5, 8.5 Hz, H-4), 3.90 (t, 1 H, *J* = 10.2, H-6$_b$), 3.83 (s, 3 H, $CH_{3\text{-COOMe}}$), 3.78 (dd, 1 H, *J* = 10.4, 8.5 Hz, H-3′′), 3.61 (td, 1 H, *J* = 9.9, 5.1 Hz, H-5), 3.56 (dd, 1 H, *J* = 10.4, 5.1 Hz, H-6a′′), 3.51 – 3.43 (m, 2 H, H-2 and H-2′′), 3.36 (dd, 1 H, *J* = 9.4, 8.5 Hz, H-4′′), 3.26 (td, 1 H, *J* = 9.9, 5.1 Hz, H-5′′), 2.72 - 2.65 (t, 1 H, *J* = 10.3 Hz, H-6b′′), 2.36 (d, 3 H, *J* = 1.1 Hz, $CH_{3\text{-4-MU}}$), 1.77 (s, 3 H, $CH_{3\text{-NAc}}$), 1.06 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 0.98 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 0.90 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 0.89 (s, 9 H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.7 (C=O$_{NAc}$), 169.3 (C=O$_{COOMe}$), 165.2, 165.1 (C=O$_{Bz}$ x2), 162.3 (C-2$_{4\text{-MU}}$), 160.9 (C=O$_{TCA}$), 159.5, 154.7, 152.3 (C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 133.6, 133.1 (CH_{arom} x2), 129.8 ($C_{q\text{-arom}}$), 129.7, 129.6 (CH_{arom} x2), 129.0 ($C_{q\text{-arom}}$), 128.6, 128.4 (CH_{arom} x2), 125.6 (C-5$_{4\text{-MU}}$), 115.2 (C-4$_{4\text{-MU}}$), 113.5 (C-6$_{4\text{-MU}}$), 112.8 (C-3$_{4\text{-MU}}$), 104.1 (C-8$_{4\text{-MU}}$), 100.4 (C-1′), 99.3 (C-1′′), 97.2 (C-1), 92.6 ($C_{q\text{-CCl3}}$), 79.3 (C-3), 77.4 (C-4′′), 76.4 (C-4), 75.7 (C-4′), 74.1 (C-5′), 73.7 (C-3′′), 72.8 (C-2′), 72.7 (C-3′), 70.4 (C-5′′), 70.1 (C-5), 66.1 (C-6), 65.0 (C-6′′), 58.2 (C-2), 57.0 (C-2′′), 53.1 ($CH_{3\text{-COOMe}}$), 27.34, 27.29, 26.9, 26.8 ($CH_{3\text{-tBu-Si}}$ x4), 23.3 ($CH_{3\text{-NAc}}$), 22.6, 22.4, 19.9, 19.7 ($C_{q\text{-tBu-Si}}$ x4), 18.6 ($CH_{3\text{-4-MU}}$); IR (neat): 3351, 2934, 2859, 1718, 1270, 1069, 827, 731, 709 cm^{-1}; HRMS Calcd for $[C_{63}H_{81}Cl_3N_2O_{21}Si_2 + Na]^+$: 1385.3828, found 1385.3839.

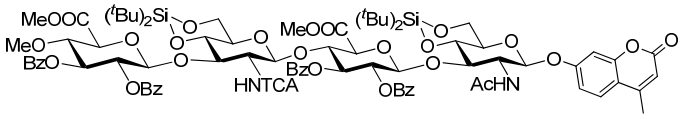


Methyl (2,3-di-*O*-benzoyl-4-*O*-methyl-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)-*α*/*β*-D-glucopyranoside) uronate (13) To a solution of hemiacetal **12** (0.75 g, 1.8 mmol, 1.0 eq) in acetone (8 mL) were Cs_2CO_3 (1.1 g, 3.5 mmol, 2.0 eq) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride[18] (0.54 mL, 3.5 mmol, 2.0 eq) added at 0 °C. After 1.5 hours at 0 °C was the mixture filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–10% EtOAc in petroleum ether) yielded the title imidate as a colorless oil in a 1:5 *α*/*β*-ratio (0.68 g, 1.1 mmol, 64%). NMR assignment for the major isomer (*β*): R_f = 0.45 (20% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 – 8.01 (m, 2 H, H_{arom}), 7.98 – 7.94 (m, 2 H, H_{arom}), 7.56 – 7.50 (m, 2 H, H_{arom}), 7.14 – 7.08 (m, 2 H, $H_{arom\text{-NPh}}$), 6.99 (t, 1 H, *J* = 7.4 Hz, $H_{arom\text{-NPh}}$), 6.78 (d, 1 H, *J* = 7.5 Hz, H-1), 6.40 (bd, 2 H, *J* = 6.1 Hz, $H_{arom\text{-NPh}}$), 6.03 (t, 1 H, *J* = 9.8 Hz, H-3), 5.47 (dd, 1 H, *J* = 10.1, 3.3 Hz, H-2), 4.52 (d, 1 H, *J* = 10.0 Hz, H-5), 3.97 (t, 1 H, *J* = 9.7 Hz, H-4), 3.87 (s, 3 H, $CH_{3\text{-COOMe}}$), 3.46 (s, 3 H, $CH_{3\text{-OMe}}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 168.4 (C=O$_{COOMe}$), 165.31, 165.25 (C=O$_{Bz}$ x2), 142.7 ($C_{q\text{-arom-NPh}}$), 133.6, 133.4, 129.9, 129.7 (CH_{arom} x4), 129.1 ($C_{q\text{-arom}}$), 128.54, 128.51, 128.48, 128.43 (CH_{arom} x4), 124.5, 124.3, 119.0 ($CH_{arom\text{-NPh}}$ x3), 92.1 (C-1), 78.7 (C-4), 72.2 (C-5), 71.3 (C-3), 70.2 (C-2), 60.6 ($CH_{3\text{-OMe}}$), 52.9 ($CH_{3\text{-COOMe}}$); IR (neat): 2959, 2925, 1718, 1451, 1261, 909, 707 cm^{-1}; HRMS Calcd for $[C_{30}H_{26}F_3NO_9 + Na]^+$: 624.1452, found 624.1451.



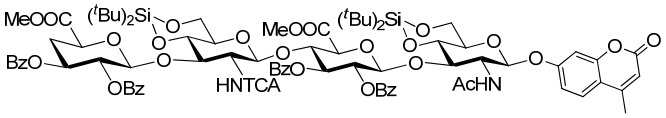
Methyl (2,3-di-*O*-benzoyl-3-deoxy-1-*O*-(*N*-[phenyl]-trifluoroacetimidoyl)-*α*/*β*-D-glucopyranoside) uronate (15) To a solution of 4-deoxy hemiacetal **14** (0.36 g, 0.73 mmol, 1.0 eq) in acetone (5 mL) were Cs_2CO_3 (0.47 g, 1.5 mmol, 2.0 eq) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride[18] (0.26 mL, 1.5 mmol, 2.0 eq) added at 0 °C. After 2 hours at 0 °C was the mixture

filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (0–5% acetone in petroleum ether) produced the title imidate as a colorless oil in a 2:3 *α/β*-ratio (0.31 g, 0.54 mmol, 73%). NMR assignment for the major isomer (*β*): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 – 7.95 (m, 4 H, H_{arom}), 7.59 – 7.49 (m, 2 H, H_{arom}), 7.46 – 7.36 (m, 4 H, H_{arom}), 7.27 (m, 1 H, $H_{arom\text{-}NPh}$), 7.14 – 7.06 (m, 2 H, $H_{arom\text{-}NPh}$), 6.81 (d, 1 H, *J* = 7.7 Hz, H-1), 6.39 (bd, 2 H, *J* = 5.0 Hz, H_{arom}), 5.83 (ddd, 1 H, *J* = 11.0, 10.8, 5.0 Hz, H-3), 5.60 (m, 1 H, H-2), 4.82 (bd, 1 H, *J* = 11.8 Hz, H-5), 3.81 (s, 3 H, $CH_{3\text{-}COOMe}$), 2.89 (m, 1 H, H-4_{eq}), 2.13 (m, 1 H, H-4_{ax}); ^{13}C NMR (101 MHz, CDCl3) δ: 169.0 (C=O_{COOMe}), 165.6, 165.3 (C=O_{Bz} x2), 142.8 ($C_{q\text{-}arom\text{-}NPh}$), 133.6, 133.4, 129.8, 129.74, 129.65 (CH_{arom} x5), 129.1, 128.8 ($C_{q\text{-}arom}$ x2), 128.6, 128.53, 128.48, 128.45, 128.41, 128.38 (CH_{arom} x6), 124.3, 124.2, 119.0 ($CH_{arom\text{-}NPh}$ x3), 92.8 (C-1), 70.4 (C-2), 68.9 (C-5), 67.6 (C-3), 52.7 ($CH_{3\text{-}COOMe}$), 32.9 (C-4); IR (neat): 2959, 2925, 1718, 1452, 1272, 1208, 1070, 1027, 777, 695 cm^{-1}; HRMS Calcd for $[C_{29}H_{24}F_3NO_8 + Na]^+$: 594.1346, found 594.1348.

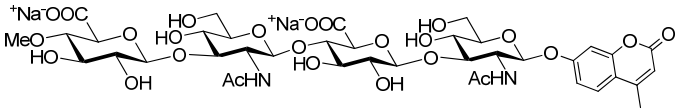


4-Methylumbelliferyl 2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-[methyl 2,3-di-*O*-benzoyl-4-*O*-{4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-methyl-*β*-D-glucopyranosyl uronate)-2-trichloroacetamido-*β*-D-glucopyranosyl}-*β*-D-glucopyranosyl uronate]-*β*-D-glucopyranoside (16) Trisaccharide acceptor **11** (0.10 g, 0.073 mmol, 1.0 eq) and imidate donor **13** (88 mg, 0.15 mmol, 2.0 eq) was coevaporated two times in anhydrous toluene and then dissolved in anhydrous DCM (2 mL). Activated molsieves (3Å) was added and the reaction mixture was stirred for one hour at room temperature, before cooling to 0 °C and addition of triflic acid (~1.0 µL, 11 µmol, 0.15 eq). The reaction was stirred for 4 hours and then quenched with triethylamine (0.1 mL, 0.73 mmol, 10 eq). The reaction mixture was transferred to an extraction funnel using DCM (20 mL) and washed with sat. aq. $NaHCO_3$ (20 mL) and brine (20 mL). The aqueous layers were extracted with DCM (20 mL) and the combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by size exclusion chromatography, followed by column chromatography (30–50% EtOAc in petroleum ether) to give the title tetrasaccharide **16** in (0.11 g, 0.062 mmol, 85% yield). R_f = 0.22 (50% EtOAc in petroleum ether); $[\alpha]_D^{22}$: + 8.3 (C = 0.157, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 – 7.90 (m, 8 H, H_{arom}), 7.53 – 7.49 (m, 4 H, H_{arom}), 7.45 (d, 1 H, *J* = 9.5 Hz, H-$5_{4\text{-}MU}$), 7.41 – 7.35 (m, 8 H, H_{arom}), 6.86 (d, 1 H, *J* = 9.5 Hz, H-$6_{4\text{-}MU}$), 6.84 (d, 1 H, *J* = 7.5 Hz, NH_{TCA}), 6.82 (s, 1 H, H-$8_{4\text{-}MU}$), 6.13 (d, 1 H, *J* = 1.0 Hz, H-$3_{4\text{-}MU}$), 5.71 (d, 1 H, *J* = 7.4 Hz, NH_{NAc}), 5.67 (d, 1 H, *J* = 8.2 Hz, H-1), 5.52 (t, 1 H, *J* = 9.1 Hz, H-3‴), 5.50 (t, 1 H, *J* = 9.1 Hz, H-3′), 5.37 (dd, 1 H, *J* = 9.1, 6.7 Hz, H-2′), 5.25 (dd, 1 H, *J* = 8.9, 6.5 Hz, H-2‴), 5.13 (d, 1 H, *J* = 6.7 Hz, H-1′), 5.07 (d, 1 H, *J* = 6.5 Hz, H-1‴), 4.99 (d, 1 H, *J* = 8.2 Hz, H-1″), 4.41 (dd, 1 H, *J* = 10.1, 8.5 Hz, H-3), 4.35 (t, 1 H, *J* = 9.1 Hz, H-4′), 4.20 – 4.14 (m, 2 H, H-6_a and H-3″), 4.06 (d, 1 H, *J* = 9.2 Hz, H-5′), 4.02 (d, 1 H, *J* = 9.0 Hz, H-5‴), 3.95 (t, 1 H, *J* = 9.0 Hz, H-4‴), 3.94 (dd, 1 H, *J* = 9.5, 8.6 Hz, H-4), 3.88 (t, 1 H, *J* = 10.3 Hz, H-6_b), 3.81 (s, 3 H, $CH_{3\text{-}COOMe}$), 3.79 (s, 3 H, $CH_{3\text{-}COOMe}$), 3.64 – 3.56 (m, 2 H, H-5 and H-6_a″), 3.51 (t, 1 H, *J* = 9.2 Hz, H-4″), 3.39 (s, 3 H, $CH_{3\text{-}OMe}$), 3.35 (m, 1 H, H-2), 3.27 – 3.13 (m, 2 H, H-2″ and H-5″), 2.69 (t, 1 H, *J* = 10.4 Hz, H-6_b″), 2.36 (s, 3 H, $CH_{3\text{-}4MU}$), 1.76 (s, 3 H, $CH_{3\text{-}NAc}$), 1.04 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.97 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.90 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.82 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.7 (C=O_{NAc}), 168.6, 168.5 (C=O_{COOMe} x2), 165.4, 165.34, 165.29, 165.1 (C=O_{Bz} x4), 161.5 (C-$2_{4\text{-}MU}$), 160.9 (C=O_{TCA}), 159.5, 154.7, 152.2 (C-$7_{4\text{-}MU}$, C-$9_{4\text{-}MU}$ and C-$10_{4\text{-}MU}$), 133.6, 133.3, 133.1, 129.9 (CH_{arom} x4), 129.8 ($C_{q\text{-}arom}$), 129.70, 129.67, 129.58 (CH_{arom} x3), 129.2, 129.08, 129.03 ($C_{q\text{-}arom}$ x3), 128.6, 128.42, 128.37, 128.33 (CH_{arom} x4), 125.6 (C-$5_{4\text{-}MU}$), 115.2 (C-$4_{4\text{-}MU}$), 113.4 (C-$6_{4\text{-}MU}$), 112.9 (C-$3_{4\text{-}MU}$), 104.3 (C-$8_{4\text{-}MU}$), 100.4 (C-1′), 99.6 (C-1‴), 98.4 (C-1″), 97.0 (C-1), 92.5 ($C_{q\text{-}CCl3}$), 79.2 (C-3), 78.4 (C-4‴), 77.6 (C-3″), 76.2 (C-4), 75.11 (C-4″), 75.08 (C-4′), 74.5 (C-5′), 74.2 (C-5‴), 74.1 (C-3‴), 73.1 (C-2‴), 72.8 (C-3′), 72.6

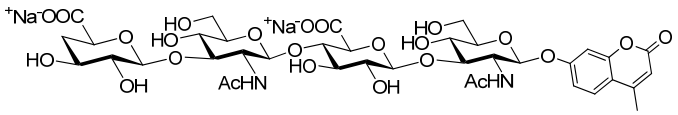
(C-2′), 70.4 (C-5), 70.2 (C-5″), 66.1 (C-6), 65.2 (C-6″), 60.4 ($CH_{3\text{-}OMe}$), 58.7 (C-2″), 57.4 (C-2), 53.1, 52.6 ($CH_{3\text{-}COOMe}$ x2), 27.3 (x2), 26.9, 26.7 ($CH_{3\text{-}tBu\text{-}Si}$ x4), 23.3 ($CH_{3\text{-}NAc}$), 22.6, 22.4, 19.9, 19.6 ($C_{q\text{-}tBu\text{-}Si}$ x4), 18.6 ($CH_{3\text{-}4\text{-}MU}$); IR (neat): 3352, 2935, 2860, 1730, 1616, 1269, 1070, 828, 710 cm^{-1}; HRMS Calcd for $[C_{85}H_{101}Cl_3N_2O_{29}Si_2 + H]^+$: 1775.5167, found 1775.5180.



4-Methylumbelliferyl 2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-[methyl 2,3-di-*O*-benzoyl-4-*O*-{4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-deoxy-*β*-D-glucopyranosyl uronate)-2-trichloroacetamido-*β*-D-glucopyranosyl}-*β*-D-glucopyranosyl uronate]-*β*-D-glucopyranoside (17) Trisaccharide acceptor **11** (0.20 g, 0.15 mmol, 1.0 eq) and imidate donor **15** (0.17 g, 0.29 mmol, 2.0 eq) was coevaporated two times in anhydrous toluene and then dissolved in anhydrous DCM (4 mL). Activated molsieves (3Å, 1.0 g) was added and the reaction mixture was stirred for one hour at room temperature. The reaction was cooled to 0 °C and triflic acid (2.0 µL, 22 µmol, 0.15 eq) was added. The reaction was stirred for 4 hours and then quenched with triethylamine (0.2 mL, 1.4 mmol, 10 eq). The reaction mixture was transferred to an extraction funnel with DCM (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (30 mL). The aqueous layers were extracted with DCM (40 mL) and the combined organics were dried $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by size exclusion followed by column chromatography (30–50 % EtOAc in petroleum ether) to give the title compound in (0.20 g, 0.11 mmol, 78% yield). R_f = 0.24 (50% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +21 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.98 – 7.89 (m, 8 H, H_{arom}), 7.57 – 7.48 (m, 4 H, H_{arom}), 7.45 – 7.33 (m, 9 H, H-$5_{4\text{-}MU}$ and H_{arom}), 7.04 (d, 1 H, *J* = 7.5 Hz, NH_{TCA}), 6.84 (dd, 1 H, *J* = 7.5, 2.1 Hz, H-$6_{4\text{-}MU}$), 6.83 (d, 1 H, *J* = 2.1 Hz, H-$8_{4\text{-}MU}$), 6.14 (d, 1 H, *J* = 1.1 Hz, H-$3_{4\text{-}MU}$), 5.74 (d, 1 H, *J* = 7.3 Hz, NH_{NAc}), 5.70 (d, 1 H, *J* = 8.2 Hz, H-1), 5.54 (t, 1 H, *J* = 8.9 Hz, H-3′), 5.38 (dd, 1 H, *J* = 8.9, 6.6 Hz, H-2′), 5.31 (dd, 1 H, *J* = 9.2, 6.6 Hz, H-2‴), 5.26 (m, 1 H, H-3‴), 5.17 (d, 1 H, *J* = 6.7 Hz, H-1′), 5.08 (d, 1 H, *J* = 6.8 Hz, H-1‴), 5.07 (d, 1 H, *J* = 8.1 Hz, H-1″), 4.42 (dd, 1 H, *J* = 10.1, 8.5 Hz, H-4′), 4.40 (t, 1 H, *J* = 9.1 Hz, H-3), 4.25 (dd, 1 H, *J* = 11.9, 2.8 Hz, H-5‴), 4.24 – 4.15 (m, 2 H, H-3″ and H-6_a), 4.10 (d, 1 H, *J* = 9.3 Hz, H-5′), 3.94 (t, 1 H, *J* = 9.0 Hz, H-4), 3.88 (t, 1 H, *J* = 10.3 Hz, H-6_b), 3.80 (s, 3 H, $CH_{3\text{-}COOMe}$), 3.79 (s, 3 H, $CH_{3\text{-}COOMe}$), 3.66 – 3.57 (m, 3 H, H-5, H-4″ and H-6″$_a$), 3.41 – 3.31 (m, 2 H, H-2 and H-2″), 3.16 (ddd, 1 H, *J* = 9.8, 9.7, 4.8 Hz, H-5″), 2.77 (t, 1 H, *J* = 10.3 Hz, H-6″$_b$), 2.66 (ddd, 1 H, *J* = 12.9, 5.2, 2.8 Hz, H-4″$_{eq}$), 2.36 (d, 3 H, *J* = 1.1 Hz, $CH_{3\text{-}4\text{-}MU}$), 2.02 (m, 1 H, H-4″$_{ax}$), 1.78 (s, 3 H, $CH_{3\text{-}NAc}$), 1.04 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.97 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.88 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.81 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.8 ($C{=}O_{NAc}$), 169.1, 168.6 ($C{=}O_{COOMe}$ x2), 165.7, 165.3, 165.2, 165.1 ($C{=}O_{Bz}$ x4), 161.6 (C-$2_{4\text{-}MU}$), 160.9 ($C{=}O_{TCA}$), 159.5, 154.7, 152.2 (C-$7_{4\text{-}MU}$, C-$9_{4\text{-}MU}$ and C-$10_{4\text{-}MU}$), 133.6, 133.3, 133.14, 133.12 (CH_{arom} x4), 129.77 ($C_{q\text{-}arom}$), 129.75, 129.68 129.56 (CH_{arom} x3), 129.3, 129.05, 129.04 ($C_{q\text{-}arom}$ x3), 128.6, 128.42, 128.35, 128.27 (CH_{arom} x4), 125.6 (C-$5_{4\text{-}MU}$), 115.2 (C-$4_{4\text{-}MU}$), 113.4 (C-$6_{4\text{-}MU}$), 112.8 (C-$3_{4\text{-}MU}$), 104.2 (C-$8_{4\text{-}MU}$), 100.3 (C-1′), 98.5 (C-1″), 98.3 (C-1‴), 96.9 (C-1), 92.5 ($C_{q\text{-}CCl3}$), 79.1 (C-3), 76.7 (C-3″), 76.0 (C-4), 75.1 (C-4′), 74.5 (C-4″), 74.1 (C-5′), 73.0 (C-3′), 72.9 (C-2‴), 72.6 (C-2′), 71.2 (C-3‴), 70.4 (C-5), 70.28 (C-5‴), 70.25 (C-5″), 66.1 (C-6), 65.2 (C-6″), 58.8 (C-2), 57.5 (C-2″), 53.1, 52.5 ($CH_{3\text{-}COOMe}$ x2), 32.4 (C-4‴), 27.3, 27.3, 26.9, 26.7 ($CH_{3\text{-}tBu\text{-}Si}$ x4), 23.3 ($CH_{3\text{-}NAc}$), 22.6, 22.4, 19.9, 19.6 ($C_{q\text{-}tBu\text{-}Si}$ x4), 18.6 ($CH_{3\text{-}4\text{-}MU}$); HRMS Calcd for $[C_{84}H_{99}Cl_3N_2O_{28}Si_2 + H]^+$: 1745.5061, found 1745.5077.



4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-[4-*O*-{2-acetamido-2-deoxy-3-*O*-(4-*O*-methyl-*β*-D-glucopyranosyl uronate)-*β*-D-glucopyranosyl}-*β*-D-glucopyranosyl uronate]-*β*-D-glucopyranoside bis ammonium salt (22) Protected tetrasaccharide **16** (18 mg, 10 μmol, 1.0 eq) was dissolved in acetic acid (2 mL) and activated zinc dust (33 mg, 0.50 mmol, 50 eq) was added three times with 2 hours interval. The reaction was then stirred at ambient temperature over night. HPLC-MS indicated about 4:1 ratio of mono-chloro residue and the fully dehalogenated product. The residue was diluted with toluene and filtered through a plug of celite. The solids were washed with some additional toluene and the residue was then concentrated *in vacuo* and dissolved in anhydrous acetone (5 mL). Potassium iodide (50 mg, 0.30 mmol, 30 eq) was added and the reaction mixure was stirred over night at ambient temperature giving full conversion to the monoiodo derivate as measured by HPLC-MS. The mixture was filtered, concentrated *in vacuo* and the remaining residue was dissolved in AcOH (2 mL) and activated zinc (33 mg, 0.5 mmol, 50 eq) was added. The reaction was stirred over night giving full conversion to the fully dechlorinated product. The solids were filtrated, washed and the eluent was concentrated *in vacuo*. The residue was dissolved in MeOH (2 mL) and sodium methoxide (1.5 μL, 10μmol, 1.0 eq) was added and the reaction was stirred under argon at room temperature over night. The completion of the reaction was monitored by HPLC-MS, before beeing quenched with acetic acid (0.2 mL). The solvents were removed *in vacuo* by coevaporation with toluene (5 mL) and the residue was then dissolved in pyridine (1 mL), before addition of hydrogenfluoride/triethylamine (6.6 μL, 40 μmol, 4 eq). The reaction was stirred for three hours at ambient temperature (the completion of the reaction was confirmed by HPLC-MS) and water (1 mL) was then added followed by sat. aq. sodium carbonate (0.5 mL). The reaction was then stirred at ambient temperature over night and was then concentrated *in vacuo*. The product was purified by HPLC according to the general procedure. The title compound was recovered as the bis ammonium salt (4.3 mg, 4.4 μmol, 43%). ^{1}H NMR (600 MHz, D_2O) δ: 7.74 (d, 1 H, *J* = 9.4 Hz, H-5$_{4\text{-MU}}$), 7.08 – 7.05 (m, 2 H, H-6$_{4\text{-MU}}$ and H-8$_{4\text{-MU}}$), 6.28 (s, 1 H, H-3$_{4\text{-MU}}$), 5.29 (d, 1 H, *J* = 8.5 Hz, H-1), 4.57 (d, 1 H, *J* = 7.9 Hz, H-1′), 4.55 (d, 1 H, *J* = 8.3 Hz, H-1′′),4.47 (d, 1 H, *J* = 7.8 Hz, H-1′′′), 4.16 (dd, 1 H, *J* = 10.5, 8.5 Hz, H-2), 3.95 (dd, 1 H, *J* = 12.1, 2.3 Hz, H-6$_a$), 3.93 – 3.89 (m, 2 H, H-6$_a$′′ and H-5′), 3.88 – 3.83 (m, 3 H, H-3, H-5′′′ and H-2′′),3.83 – 3.78 (m, 2 H, H-6$_a$ and H-4′), 3.76 (dd, 1 H, *J* = 12.4, 5.2 Hz, H-6$_a$′′), 3.73 – 3.69 (m, 2 H, H-4 and H-5), 3.67 (m, 1 H, H-3′′), 3.65 (m, 1 H, H-3′), 3.55 (t, 2 H, *J* = 9.3 Hz, H-4′′ and H-3′′′), 3.50 – 3.46 (m, 4 H, H-5′′ and CH$_{3\text{-OMe}}$), 3.39 (dd, 1 H, *J* = 9.5, 7.9 Hz, H-2′), 3.33 (dd, *J* = 9.5, 7.9 Hz, 1 H, H-2′′′), 3.30 (t, *J* = 9.5 Hz, 1 H, H-4′′′), 2.45 (s, 3 H, CH$_{3\text{-4MU}}$), 2.00 (s, 3 H, CH$_{3\text{-NAc}}$), 1.99 (s, 3 H, CH$_{3\text{-NAc}}$); ^{13}C NMR (151 MHz, D_2O) δ: 175.9, 175.8 (C=O$_{NAc}$ x2), 174.7, 173.4 (C=O$_{COO\text{-}NH4+}$ x2), 165.7, 160.4, 157.3, 155.0 (C-2$_{4\text{-MU}}$, C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 127.7 (C-5$_{4\text{-MU}}$), 116.6 (C-4$_{4\text{-MU}}$), 114.9 (C-6$_{4\text{-MU}}$), 112.4 (C-3$_{4\text{-MU}}$), 104.7 (C-8$_{4\text{-MU}}$), 104.0 (C-1′′′), 103.6 (C-1′), 101.9 (C-1′′), 99.6 (C-1), 83.5 (C-5), 82.9 (C-3), 82.7 (C-4′′), 81.0 (C-4′), 76.7 (C-5), 76.3 (C-5′′), 75.69 (C-3′′′), 75.65 (C-5′), 75.1 (C-5′′′), 74.6 (C-3′), 73.5 (C-2′′′), 73.2 (C-2′), 69.2 (C-4′′), 69.1 (C-4), 61.43 (C-6′′), 61.35 (C-6), 61.1 (CH$_{3\text{-OMe}}$), 55.24 (C-2), 55.19 (C-2′′), 23.4, 23.1 (CH$_{3\text{-NAc}}$ x2), 19.0 (CH$_{3\text{-4-MU}}$); ESI-MS *m/z*: 949.3 [M + H]$^+$, 971.3 [M + Na]$^+$.



4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-[4-*O*-{2-acetamido-2-deoxy-3-*O*-(4-deoxy-*β*-D-glucopyranosyl uronate)-*β*-D-glucopyranosyl}-*β*-D-glucopyranosyl uronate]-*β*-D-glucopyranoside bis ammonium salt (23) Protected tetrasaccharide **17** (75 mg, 42 µmol, 1.0 eq) was dissolved in acetic acid (5 mL) and activated zinc dust (0.14 g, 2.1 mmol, 50 eq) were added three times with 2 hours interval. The reaction was then stirred at ambient temperature over night. HPLC-MS indicated about 5:2 ratio of mono-chloro residue and the fully dehalogenated product. The residue was diluted with toluene and filtered through a plug of celite. The solids were washed with some additional toluene and the residue was then concentrated *in vacuo* and dissolved in anhydrous acetone (5 mL). Potassium iodide (0.21 g, 1.3 mmol, 30 eq) was added and the reaction mixure was stirred over night at ambient temperature giving full conversion to the monoiodo derivate as measured by HPLC-MS. The mixture was filtered, concentrated *in vacuo* and the remaining residue was dissolved in AcOH (5 mL) and activated zinc (138 mg, 2.1 mmol, 50 eq) was added. The reaction was stirred over night giving full conversion to the fully dechlorinated product. The solids were filtrated, washed and the eluent was concentrated *in vacuo*. The residue was dissolved in MeOH (5 mL) and sodium methoxide (29 µL, 44 µmol, 1.0 eq) was added and the reaction was stirred under argon at room temperature over night. The completion of the reaction was monitored by HPLC-MS, before beeing quenched with acetic acid (0.2 mL). The solvents were removed *in vacuo* by coevaporation with toluene (5 mL) and the residue was then dissolved in pyridine (3 mL), before addition of hydrogen fluoride/triethylamine (29 µL, 17 mmol, 4 eq). The reaction was stirred for three hours at ambient temperature (the completion of the reaction was confirmed by HPLC-MS) and water (3 mL) was added followed by sat. aq. sodium carbonate (1.5 mL). The reaction was then stirred at ambient temperature over night and was then concentrated *in vacuo*. The product was purified by HPLC according to the general procedure. The title compound was recovered as the bis ammonium salt (24 mg, 26 µmol, 60%). ^{1}H NMR (600 MHz, D_2O) δ: 7.60 (d, 1 H, *J* = 8.8 Hz, H-5$_{4\text{-MU}}$), 6.99 (dd, 1 H, *J* = 8.8, 2.5 Hz, H-6$_{4\text{-MU}}$), 6.93 (d, 1 H, *J* = 2.5 Hz, H-8$_{4\text{-MU}}$), 6.16 (s, 1 H, H-3$_{4\text{-MU}}$), 5.25 (d, 1 H, *J* = 8.5 Hz, H-1), 4.62 (d, 1 H, *J* = 7.9 Hz, H-1′), 4.58 (d, 1 H, *J* = 8.4 Hz, H-1″), 4.42 (d, 1 H, *J* = 7.8 Hz, H-1‴), 4.30 (dd, 1 H, *J* = 12.3, 2.4 Hz, H-5‴), 4.16 (t, 1 H, *J* = 9.4 Hz, H-2), 4.02 (d, 1 H, *J* = 9.7 Hz, H-5′), 3.95 (d, 1 H, *J* = 12.1 Hz, H-6$_a$), 3.93 – 3.87 (m, 2 H, H-6$_a$″ and H-3), 3.86 – 3.80 (m, 3 H, H-2″, H-4′ and H-6$_b$), 3.79 – 3.72 (m, 3 H, H-3‴, H-6$_b$″ and H-3″), 3.71 – 3.67 (m, 2 H, H-4 and H-5), 3.65 (m, 1 H, H-3′), 3.56 (dd, 1 H, *J* = 11.9, 6.4 Hz, H-4″), 3.49 (m, 1 H, H-5″), 3.40 (t, 1 H, *J* = 8.7 Hz, H-2′), 3.21 (t, 1 H, *J* = 8.5 Hz, H-2‴), 2.39 – 2.34 (m, 4 H, H-4$_{eq}$‴ and CH$_{3\text{-}4\text{MU}}$), 2.01 (s, 3 H, CH$_{3\text{-NAc}}$), 1.99 (s, 3 H, CH$_{3\text{-NAc}}$), 1.64 (q, 1 H, H-4$_{ax}$‴); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 175.82, 175.76 (C=O$_{NAc}$ x2), 174.4, 172.3 (C=O$_{COO\text{-}NH4+}$ x2), 165.4, 160.3, 157.0, 154.7 (C-2$_{4\text{-MU}}$, C-7$_{4\text{-MU}}$, C-9$_{4\text{-MU}}$ and C-10$_{4\text{-MU}}$), 127.6 (C-5$_{4\text{-MU}}$), 116.3 (C-4$_{4\text{-MU}}$), 114.9 (C-6$_{4\text{-MU}}$), 112.3 (C-3$_{4\text{-MU}}$), 104.6 (C-8$_{4\text{-MU}}$), 104.0 (C-1‴), 103.8 (C-1′), 102.1 (C-1″), 99.5 (C-1), 84.1 (C-3″), 83.0 (C-3), 81.1 (C-4′), 76.7 (C-5), 76.3 (C-5″), 75.0 (C-2‴), 74.6 (C-3′), 74.5 (C-5′), 73.1 (C-2′), 71.2 (C-5‴), 70.7 (C-3‴), 69.4 (C-4″), 69.0 (C-4), 61.5 (C-6″), 61.3 (C-6), 55.20 (C-2), 55.16 (C-2″), 36.2 (C-4‴), 23.3, 23.2 (CH$_{3\text{-NAc}}$ x2), 18.9 (CH$_{3\text{-}4\text{-MU}}$). ESI-MS *m/z*: 919.3 $[M + H]^+$, 941.3 $[M + Na]^+$.

References and footnotes

1. Zinterhofer, L.; Schuttringer, G. *Clin. Chem.* **1976,** *22* (10), 1631-1634.
2. Stern, R. *Eur. J. Cell Biol.* **2004,** *83* (7), 317-325.
3. Krag, J.; Christiansen, M. S.; Petersen, J. G.; Jensen, H. H. *Carbohydr. Res. 345* (7), 872-879.

4. Slaghek, T. M.; Hypponen, T. K.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron Lett.* **1993,** *34* (49), 7939-7942.
5. Slaghek, T. M.; Nakahara, Y.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1994,** *255*, 61-85.
6. Huang, L. J.; Huang, X. F. *Chem.–Eur. J.* **2007,** *13* (2), 529-540.
7. Yeung, B. K. S.; Hill, D. C.; Janicka, M.; Petillo, P. A. *Org. Lett.* **2000,** *2* (9), 1279-1282.
8. Blatter, G.; Jacquinet, J. C. *Carbohydr. Res.* **1996,** *288*, 109-125.
9. Dinkelaar, J.; Codee, J. D. C.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2007,** *72* (15), 5737-5742.
10. Dinkelaar, J.; Gold, H.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009,** *74* (11), 4208-4216.
11. Blatter, G.; Beau, J. M.; Jacquinet, J. C. *Carbohydr. Res.* **1994,** *260* (2), 189-202.
12. Takano, Y.; Kojima, N.; Nakahara, Y.; Hojo, H.; Nakahara, Y. *Tetrahedron* **2003,** *59* (42), 8415-8427.
13. Hanashima, S.; Castagner, B.; Esposito, D.; Nokami, T.; Seeberger, P. H. *Org. Lett.* **2007,** *9* (9), 1777-1779.
14. Valerio, S.; Pastore, A.; Adinolfi, M.; Iadonisi, A. *J. Org. Chem.* **2008,** *73* (12), 4496-4503.
15. Dinkelaar, J.; Witte, M. D.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Carbohydr. Res.* **2006,** *341* (10), 1723-1729.
16. El-Abadla, N.; Lampilas, M.; Hennig, L.; Findeisen, M.; Welzel, P.; Muller, D.; Markus, A.; van Heijenoort, J. *Tetrahedron* **1999,** *55* (3), 699-722.
17. Vibert, A.; Lopin-Bon, C.; Jacquinet, J. C. *Tetrahedron Lett.* **2010,** *51* (14), 1867-1869.
18. Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K. *J. Org. Chem.* **1993,** *58* (1), 32-35.

Chapter 6

Synthesis of Sugar Nucleotides – A Phosphoramidite Approach

Introduction

Glycans are abundant in all biological systems and their molecular structure enables a high degree of diversity, more so than that of either proteins or nucleic acids.[1] The biosynthesis of these ubiquitous and structurally diverse biopolymers is catalyzed by glycosyltransferases,[2] also known as Leloir enzymes.[3] Based on sequence similarities, 92 families of glycosyltransferases can be distinguished.[4,5] These enzymes transfer a glycoside in a regio- and stereoselective manner to acceptors such as carbohydrates, lipids or proteins. Glycosyltransferases utilise activated glycosides in the form of sugar nucleotides as glycosyl donors. Sugar nucleotides are structurally composed of a sugar moiety equipped with a nucleoside mono- or diphosphate (Figure 1). While eukaryotic glycosyltransferases employ a very limited number of sugar nucleotides as glycosyl donors, the structures of nucleoside diphosphate sugars in prokaryotes are both numerous and diverse.[6]

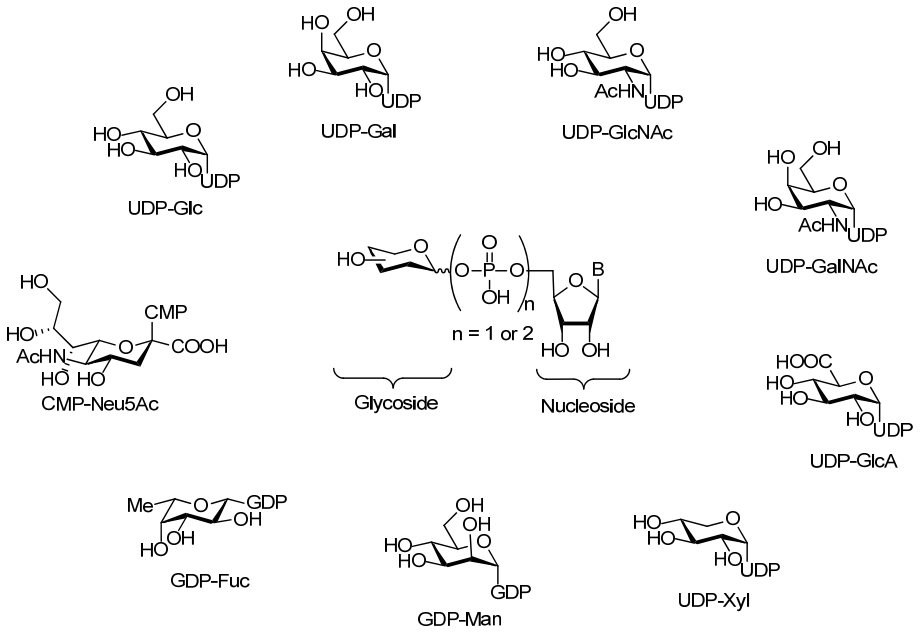


Figure 1. General structure of sugar nucleotides and the known eukaryotic sugar nucleotides.

Advances in engineering of glycosyltransferases demonstrate that these enzymes may be used for synthesis of relevant oligosaccharides[7] or even modification (i.e. glycosylation) of low molecular weight organics, which are important for the pharmaceutical industry.[8] Furthermore, synthesis of labeled or orthogonally tagged sugar-nucleotides will aid the efforts in glycomics.[9-11] However, a careful design is necessary to ensure that modification of the sugar nucleotide will not affect its primary function, to serve as a substrate for the glycosyltransferase.[12,13]

A major bottleneck in this research area is the availability of suitable sugar nucleotides and key intermediates, such as sugar-1-phosphates.[14] Both enzymatic and synthetic methodologies that make sufficient quantities of the desired sugar-nucleotides available are of great importance. Nature's own biosynthetic machinery can be utilized for the formation of sugar nucleotides and represents a good methodology for the synthesis of non-modified (i.e. naturally occurring) sugar nucleotides.[15] Advantages of enzymatic synthesis are not only represented by high stereoselectivity, but also by a relatively easy scale-up. Synthesis of modified sugar nucleotides has been accomplished by enzymatic routes, although lower yields are obtained compared to the natural substrates.[14]

The many applications and importance of sugar nucleotides have encouraged the development of a variety of methodologies for their assembly. The key step in the synthesis of nucleoside diphosphate sugars is the introduction of the pyrophosphate (diphosphate) functionality between the C5′-OH position of a nucleoside and the anomeric hydroxyl of a saccharide unit. The pioneering work with chemical synthesis of sugar nucleotides was done by Khorana and Moffatt in the late 1950s (Figure 2).[16]

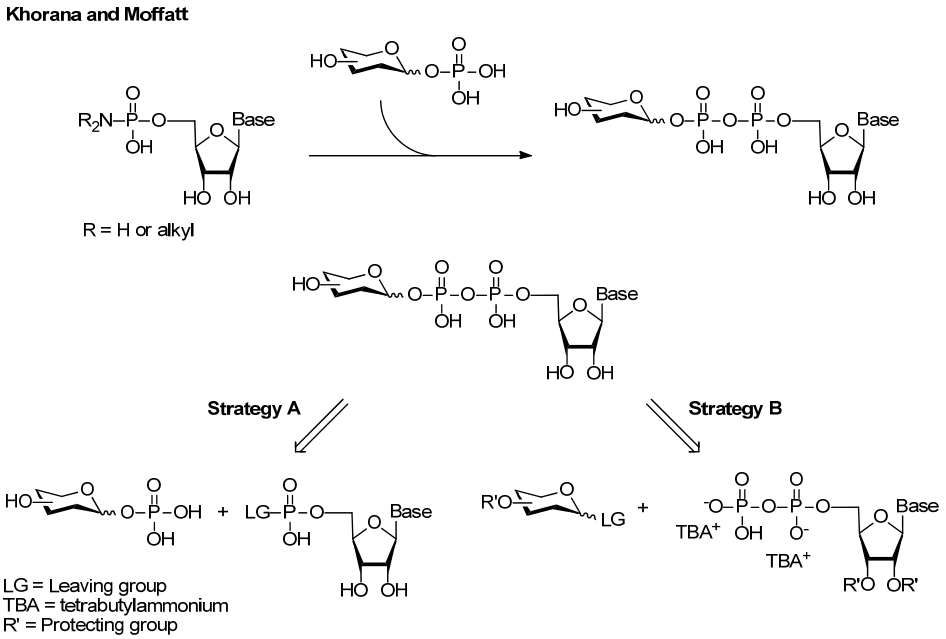


Figure 2. Synthetic strategies for the assembly of sugar nucleotides.

Their original method entailed the synthesis of an activated nucleoside phosphoramidate, which upon condensation with a sugar phosphate produced the target sugar nucleotide. The Khorana-Moffatt methodology is still the most widely pursued methodology for chemical synthesis of sugar nucleotides, with phosphoromorpholidates[17] as the preferred (P^V) condensation partner. Improvements of this method in terms of reaction time and yield have been reported by the utilization of catalysts, such as tetrazole.[18] A recent review disclosed most of the published methodologies, which in large can be divided in two distinctive strategies (Figure 2).[19] Strategy A is represented by methodologies similar to the Khorana-Moffatt procedure and are defined by the condensation of a stereo chemically predefined sugar phosphate with an activated phosphorous (P^V) nucleotide. Strategy B comprises the reaction of a protected glycosyl donor, such as a halogenide, with the tetrabutylammonium salt of a nucleoside 5'-diphosphate.[20-22] The latter approach is often hampered by a low anomeric diastereoselectivity, although exceptions have been reported in the literature.[23]

This chapter describes the development of a novel strategy for the synthesis of sugar nucleotides. The efficient and powerful phosphorylating property of phosphoramidite (P^{III}) chemistry, commonly used in the automated synthesis of oligonucleotides,[24] was explored for application in the synthesis of nucleoside diphosphate sugars. In a one-pot procedure, condensation of a phosphoramidite (P^{III}) and a phosphate monoester is followed by oxidation and deprotection to give the target sugar nucleotide.

Results and Discussion

The new methodology for the synthesis of sugar nucleotides was investigated by the preparation of a set of GlcNAc-UDP analogues (Figure 3). The modified sugar nucleotides **2** and **3** were selected by their virtue of being potential chain terminators in enzyme mediated polymerization reactions. For instance, hyaluronic acid (HYA) is assembled using a hyaluronan synthase (HAS), UDP-GlcNAc, (**1**) and UDP-glucuronic acid (UDP-GlcA).[25] Incorporation of an *N*-acetylglucosamine in which the C3-OH function is capped with a methyl group (**2**) or removed (**3**) would prohibit chain elongation at that position. Thus, it is expected that the length of the HYA oligomer can be tuned by the use of **2** or **3** as additional substrates in the HAS reaction. Recently, the group of Fairbanks reported the potential of C3″-OH modified UDP-glucose derivatives as a new class of inhibitors of the biosynthesis of essential oligosaccharides of pathogens.[26]

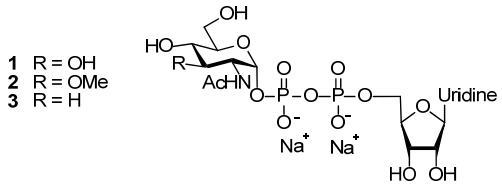
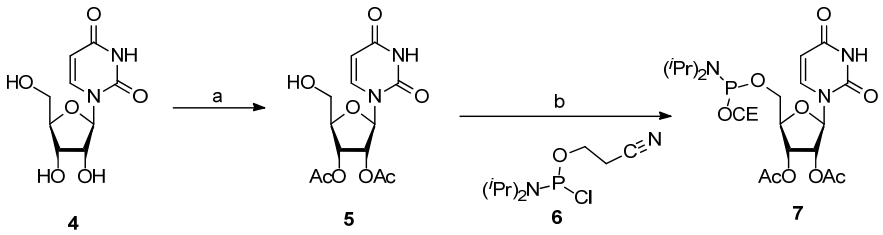


Figure 3. GlcNAc-UDP **1**, and GlcNAc-UDP analogues **2** and **3**.

Uridine phosphoramidite **7**[27] (Scheme 1) was prepared according to literature procedure with minor modifications. 2′,3′-*O*-Diacetyluridine **5** was synthesized in a straightforward fashion with only work-up between steps. The primary alcohol **5** was then phosphitylated with 2-cyanoethoxy-*N*,*N*-diisopropylamidochloridophosphite **6**[28], giving uridine phosphoramidite **7**. The 2-cyanoethyl group was selected as a phosphite protective group because of its easy removal under anhydrous, basic conditions in the phosphotriester stage.

Scheme 1. Synthesis of uridine phosphoramidite **7**.

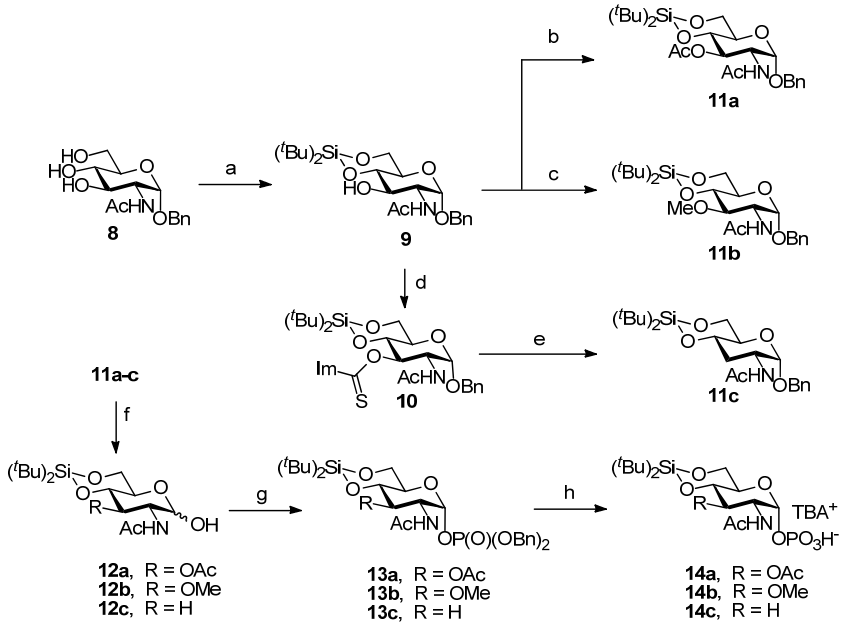


Reagents and conditions: [a] i) Tritylchloride, DCE/pyridine, 50 °C, 18 h.; ii) Ac_2O, DCE/pyridine, r.t., 18 h.; iii) AcOH aq., reflux, 3 h., 93% over three steps. [b] **6**, DiPEA, DCM, r.t., 1h., 99%.

GlcNAc-*α*-1-phosphate **14a** (Scheme 2) was synthesized, starting from the known benzyl 2-acetamido-2-deoxy-*α*-D-glucoside **8**[12], in 84% total yield by the following five step sequence of events: The 4,6-diol system of GlcNAc **8** was protected with the di-*tert*-butylsilanediyl group, which greatly enhances the solubility of GlcNAc

derivatives. The remaining hydroxyl of **9** was acetylated giving **11a**, after which the anomeric benzyl group was removed by treatment with $Pd(OH)_2$ and hydrogen gas to produce hemiacetal **12a**. The anomeric phosphate was stereoselectively introduced by treatment of **12a** with LDA at −78 °C, followed by addition of tetrabenzyl pyrophosphate (TBPP) to give the *α*-dibenzyl-phosphotriester **13a**. Hydrogenolysis of the benzyl groups in **13a** produced the free GlcNAc-*α*-phosphate, which was further transformed into its tetrabutylammonium (TBA) salt **14a** *via* addition of tetrabutylammonium hydroxide and lyophilization. Additionally, C3-capped GlcNAc-*α*-phosphates **14b** and **14c** were synthesized in a straightforward fashion (Scheme 2). The C3-*O*-methyl group in **11b** was introduced *via* the $BF_3{\cdot}OEt_2$ catalyzed methylation with TMS-diazomethane as methyl source, previously described in **Chapter 4**. The C3-deoxy derivative **11c** was synthesized by initial transformation of GlcNAc **9** to the corresponding imidazole thiocarbonyl derivative **10**, followed by radical deoxygenation. GlcNAc-*α*-phosphates **14b** and **14c** were then produced from **11b** and **11c** respectively, following the same reaction sequence as described for the transformation of **11a** to **14a**.

Scheme 2. Synthesis of sugar phosphates **14a-c**.

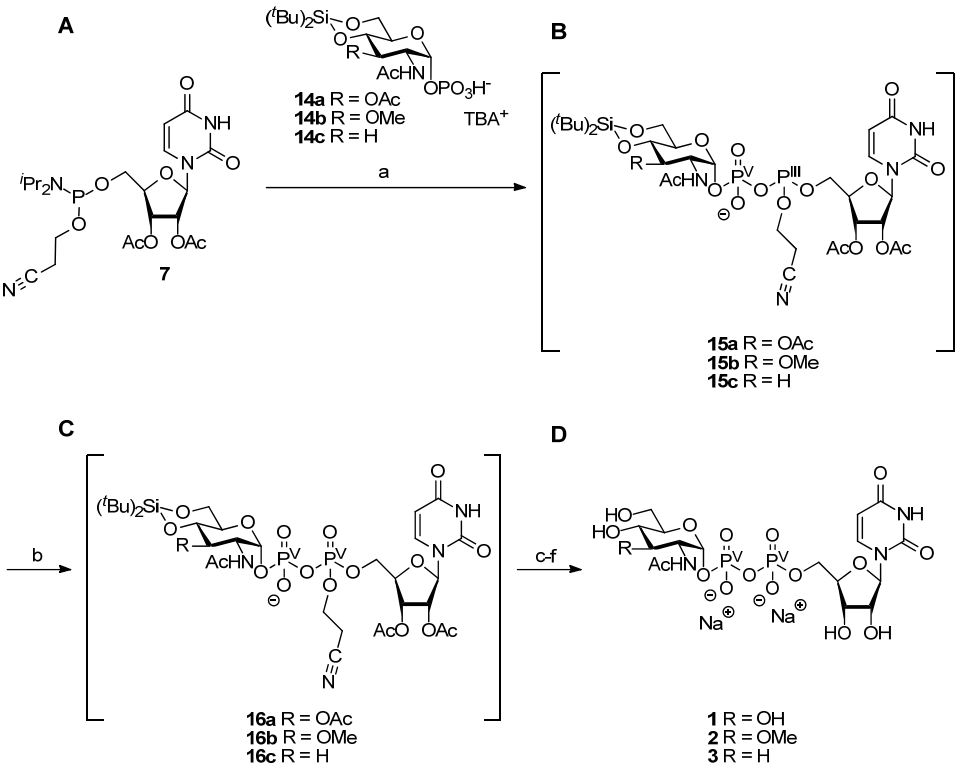


Reagents and conditions: [a] $^tBu_2Si(OTf)_2$, pyridine, DMF, −40 °C, 45 min., 94%. [b] Ac_2O, pyridine, r.t., 3 h, 99%. [c] $TMSCHN_2$, $BF_3{\cdot}Et_2O$, DCM, −40 °C, 45 min., 62%. [d] Im_2CS, toluene, 90°, 18 h, 99%. [e] $^nBu_3SnH$, AIBN, toluene, 95 °C, 4h, 94%. [f] $Pd(OH)_2$, H_2, dioxane/MeOH, r.t., 20 h, **12a**: 93%; **12b**: 97%; **12c**: 98%. [g] LDA, TBPP, THF, −78 °C to r.t., 3 h, **13a**: 98%; **13b**: 94%; **13c**: 84%. [h] Pd/C 10%, MeOH, r.t., 3 h, then TBA-OH aq. 50%, **14a**: 99%; **14b**: 99%; **14c**: 97%.

With sugar phosphate **14a** and phosphoramidite **7** in hand, the pyrophosphate formation was examined (Scheme 3). Optimization of the reaction parameters could be performed by following the reaction progress with ^{31}P NMR spectroscopy, using an

NMR capillary filled with acetone-d_6. The activator dicyanoimidazole[29] and phosphate monoester **14a** (δ: −1.1 ppm) were simultaneously added to a solution of phosphoramidite **7** (δ: 149.5, 149.4 ppm) in acetonitrile. Within 30 minutes at room temperature, complete disappearance of phosphoramidite **7** and formation of two diastereomeric phosphate-phosphite intermediates **15a** [δ: 130.3 (d, *J* = 5.8 Hz), 128.3 (d, *J* = 4.5 Hz), −10.9 (d, *J* = 4.5 Hz), −11.0 (d, *J* = 5.8 Hz) ppm] were observed. Subsequent oxidation with anhydrous *tert*-butylhydroperoxide[30] gave the diastereoisomeric cyanoethyl-protected pyrophosphate **16a** [δ: −10.7 (d, *J* = 16.2 Hz), −11.4 (d, *J* = 17.8 Hz), −13.5 (d, *J* = 17.8 Hz), −13.6 (d, *J* = 16.2 Hz) ppm].

Scheme 3. One-pot reaction for the synthesis of GlcNAc-UDP derivates **1**–**3**.



Reagents and conditions: [a] DCI, **14a**, **14b** or **14c**, MeCN, r.t., 30 min.; [b] ^{t}BuOOH, r.t., 30 min.; [c] DBU, r.t., 30 min.; [d] $(HF)_3{\cdot}Et_3N$, Et_3N, r.t., 30 min.; [e] NH_4OH aq., r.t., 18 h, [f] Ion-exchange purification, Amberlite-Na+, lyophilization, **1**: 76%; **2**: 63%; **3**: 71% (from **7**).

The protective groups were immediately removed by a three-step procedure. First the 2-cyanoethyl group was removed by treatment with anhydrous DBU, followed by addition of $(HF)_3{\cdot}Et_3N$ to deblock the di-*tert*-butylsilylene group and ammonium hydroxide to unmask the remaining alcohol functions. Purification of the crude product mixture by ion-exchange chromatography and transformation to its sodium salt by application of Amberlite-Na^+ produced the pure GlcNAc diphosphate

nucleoside **1** [δ: −10.8 (d, *J* = 21.1 Hz), −12.5 (d, *J* = 21.1 Hz) ppm] in 76% yield. The ^{31}P-NMR spectra that were acquired during the course of the reaction are depicted in Figure 4. Noteworthy is that condensation of **7** with the glycosyl phosphate in its free acid form did not produce the intermediate P^{V}–P^{III} intermediate **15a** and that the amount of tetrabutylammonium counter ion, affected the rate and overall conversion to **15a**. It was determined that the use of one equivalent of TBA counter ion was optimal for the condensation and the efficiency was especially diminished with use of more than one equivalent. Using the optimized conditions GlcNAc-UDP analogues **2** and **3** were prepared in 63% and 71% yield respectively.

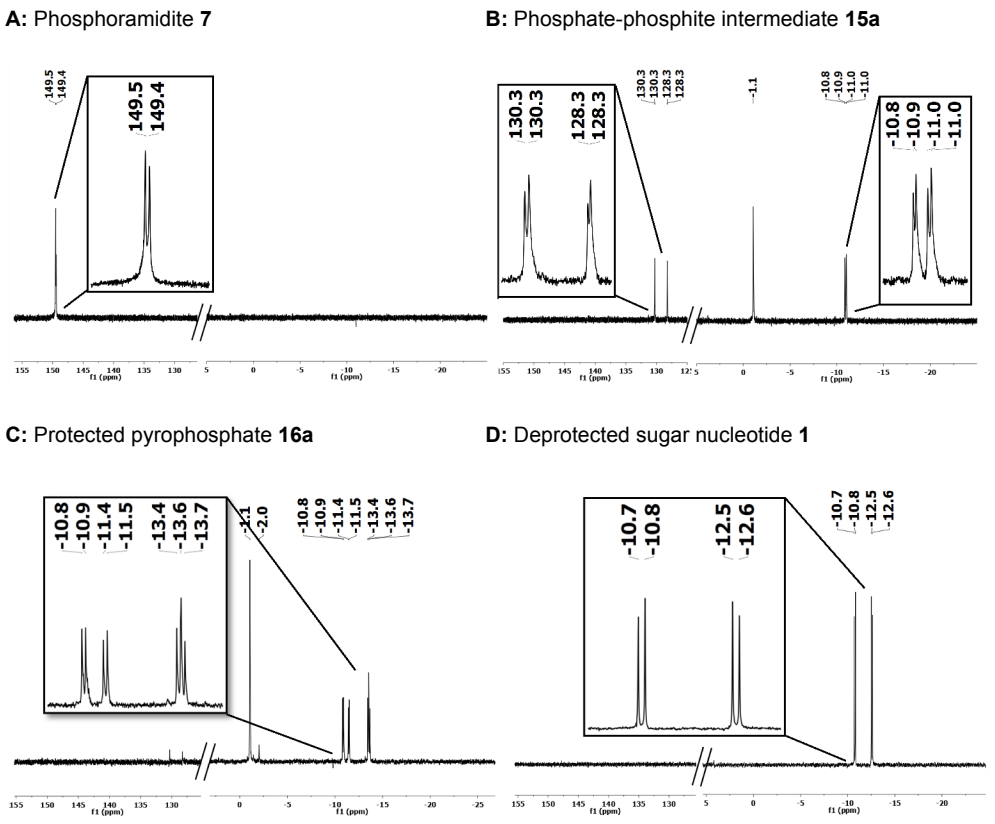


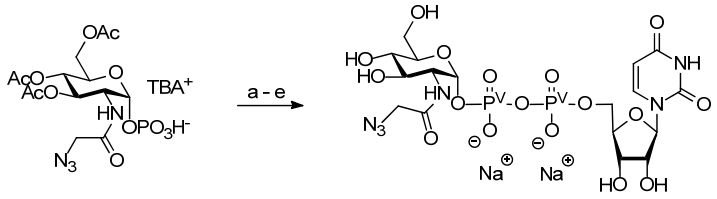
Figure 4. ^{31}P-NMR of the reaction progress towards product **1**. **A:** Phosphoramidite **7**; **B:** Activation and reaction with the sugar-*α*-phosphate forming **15a**; **C:** Oxidation forming protected pyrophosphate **16a**; **D:** Deprotection and purification giving pure GlcNAc-PPU **1**.

With the successful synthesis of GlcNAc-PPU derivates **1**–**3**, synthesis of a nucleoside diphosphate *N*-azidoacetyl glucosamine derivate **18** was investigated (Scheme 4). This azido-modified sugar nucleotide is utilized in the "bio-orthogonal chemical reporter" strategy to probe glycan patterns in living cells.[31] Introduction of a phosphate to the anomeric position of an *N*-azidoacetyl glucosamine is problematic. Reductive deprotection of common phosphotriester protective groups (i.e. benzyl)

would also reduce the azide functionality. Vocadlo *et al.* have reported a synthesis of **18** in which the anomeric phosphate was introduced as an *α*-diallyl-phosphotriester.[31] In a one-pot procedure deallylation of the anomeric phosphotriester with palladium tetrakis triphenyl phosphine [$Pd(PPh_3)_4$], followed by *in situ* condensation of the free sugar-*α*-phosphate with uridine phosphoro-*N*-methylimidazolide[32] produced the target GlcNAz-UDP **18** in 32% yield.[33]

Application of the phosphoramidite method for the synthesis of **18** requires the availability of a GlcNAz-*α*-phosphate, which first was synthesized according to the method of Vocadlo *et al.*[31] and then converted into its tetrabutylammonium salt **17** (Scheme 4). The sugar-*α*-phosphate mono-tetrabutylammonium salt **17** was then condensed with uridine phosphoramidite **7** using the optimized conditions described for the synthesis of **1**-**3**, giving **18** in 63% yield after deprotection and purification.

Scheme 4. Synthesis of GlcNAz diphosphate nucleoside **18**.



17 **18**

Reagents and conditions: [a] **7**, DCI, MeCN, r.t., 30 min.; [b] ^{t}BuOOH, r.t., 30 min.; [c] DBU, r.t., 30 min.; [d] Et_3N/MeOH/water 2:2:1, r.t., 18 h, [e] Ion-exchange purification, Amberlite-Na^+, lyophilization, 63%.

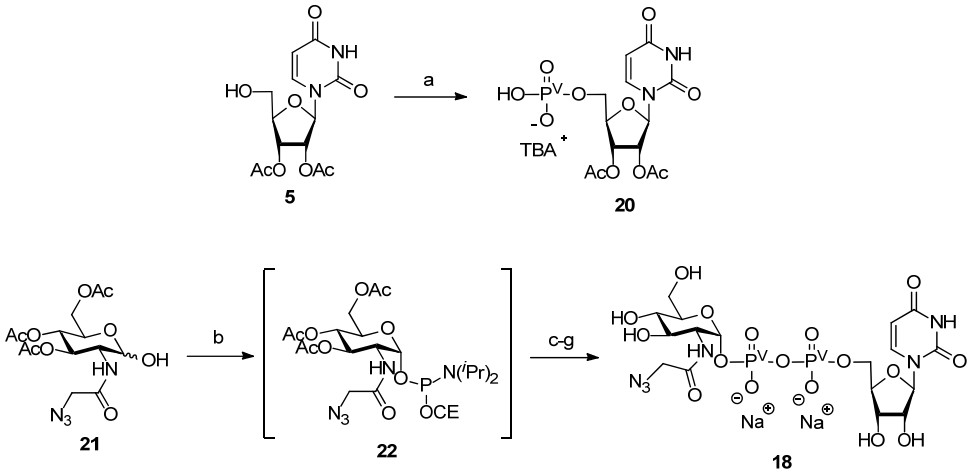
Unfortunately, the overall efficiency of the synthesis of **18** is hampered by a low yield for the formation of the GlcNAz-*α*-phosphate. In the method by Vocadlo *et al.*[31] a Staudinger reaction between the azide functionality and the triphenylphosphine ligands of the palladium catalyst may contribute to a modest yield in the formation of the GlcNAz-*α*-phosphate. Formation of the resulting aminoacetyl glucosamine-*α*-phosphate was identified by ion-exchange chromatography and MS analysis.

Although azides are known to react with numerous P^{III}-species, including phosphites,[34] it is noteworthy that the azide functionality may remain unaffected by the presence of either the phosphoramidite or any of the other intermediate P^{III}-species in the reaction mixture (Scheme 4).

In an attempt to overcome the drawback of the synthesis of GlcNAz-*α*-phosphate, a reversed version of the pyrophosphate formation, in which uridine phosphate **20** is reacted with GlcNAz-*α*-phosphoramidite **22** was investigated (Scheme 5). Uridine phosphate was initially synthesized from 2′,3′-di-*O*-acetyl uridine **5** by application of di(*p*-methoxybenzyl)-*N*,*N*-diisopropylphosphoramidite,[35] followed by transformation into its TBA-salt **20**. GlcNAz hemiacetal **21** was then phosphitylated using *n*-BuLi and phosphoramidochloridite **6**[28], giving almost exclusive formation of the GlcNAz-*α*-phosphoramidite **22** as confirmed by ^{31}P NMR. This phosphoramidite was not isolated,[36,37] but immediately implemented in the optimized procedure for the

pyrophosphate formation. Thus, GlcNAz-α-phosphoramidite was *in situ* condensed[38] with the tetrabutylammonium salt of 2′,3′-di-*O*-acetyluridine-5-phosphate **20** under the influence of DCI. Oxidation of the intermediate phosphite-phosphate intermediate and removal of protecting groups produced the crude sugar nucleotide **18**. Purification of the nucleoside diphosphate azido-sugar by ion-exchange chromatography was unsuccessful due to co-elution of the product with uridine phosphate. Crude **18** was therefore purified by size-exclusion chromatography followed by RP-HPLC, producing the required GlcNAz-α-diphosphate nucleoside **18** in 42% overall yield, starting from the GlcNAz hemiacetal **21** using a one-pot procedure.

Scheme 5. Synthesis of uridine phosphate **20** and a one-pot synthesis of GlcNAz-PPU **18**.



Reagents and conditions: [a] i) $(PMB)_2PN(^iPr)_2$, DCI, r.t., 30 min., then ^{t}BuOOH, r.t., 30 min.; iii) TFA (neat), 0 °C; iv) TBA-OH aq. 50%, 34% (from **5**). [b] *n*-BuLi, **6**, THF, −78 °C to r.t.; [c] i) DCI, MeCN, r.t., 30 min.; [d] ^{t}BuOOH, r.t., 30 min.; [e] DBU, r.t., 30 min.; [f] Et_3N, MeOH, water 2:2:1, r.t., 18 h, [g] Size-exclusion, RP-HPLC, lyophilization, Amberlite Na^+, 42% yield.

Conclusion

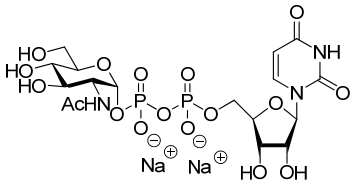
An efficient, robust and rapid one-pot procedure for the synthesis of sugar nucleotides using easily accessible nucleoside phosphoramidites and sugar phosphates was developed. The utilized phosphoramidites were shown to be powerful phosphorylating agents in the formation of the pyrophosphate moiety. This procedure represents the first methodology in which a P^{III}-species functions as an electrophile for the condensation with a sugar phosphate. Additionally, the methodology was extended, providing an easy access to the valuable and otherwise difficult to synthesize, GlcNAz-PPU. These procedures represent an attractive alternative to existing methods and are a valuable asset for future research in glycobiology.

Experimental section

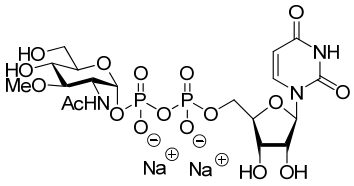
General Procedures and Material: Commercially available reagents and solvents (Acros, Fluka or Merck) were used as received unless stated otherwise. DCM and THF were freshly distilled, before use, over P_2O_5 and Na/benzophenone respectively. Acetonitrile (DNA reagent grade, Biosolve) was stored over molecular sieves (3Å) prior to use. Triethylamine was destilled over calcium hydride and stored over KOH. Compound **6** was synthesized according to literature procedures.[28] Traces of water was removed from starting compounds by coevaporation with MeCN, DCE and/or toluene. All moisture sensitive reactions were performed under an atmosphere of argon. Molecular sieves 3Å were flamedried prior to use. Liquid column chromatography was performed using forced flow of the indicated solvent systems on Screening Devices Silica gel 60 (40-63 μm mesh). Size-exclusion chromatography was performed on Sephadex LH20 (eluent MeOH/DCM, 1:1). Analytical TLC was performed on aluminium sheets, pre-coated with silica gel (Merck, silica gel 60, F_{254}). Compounds were visualized with UV absorption (245 nm), by spraying with either 20% H_2SO_4 in ethanol, or ammonium molybdate/cerium sulphate solution [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_6{\cdot}2H_2O$ (10 g/L), 10% sulphuric acid in ethanol], or phosphormolybdic acid in EtOH (150 g/L) followed by charring (~150 °C) or by spraying with an aqueous solution of potassium permanganate [$KMnO_4$ (20 g/L), K_2CO_3 (10 g/L)]. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter (Sodium D-line, λ = 589 nm). ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 (^1H), 100.6 (^{13}C) and 162.0 (^{31}P) MHz respectively. Chemical shifts are reported as δ values (ppm) and directly referenced to TMS (0.00 ppm) in $CDCl_3$ or indirectly referenced to H_3PO_4 (0.00 ppm) in D_2O *via* the solvent residual signal. Coupling constants (*J*) are given in Hz and all ^{13}C spectra are proton decoupled. NMR assignments were made using COSY and HSQC and in some cases TOCSY experiments. LC-MS analyses were performed on a LCQ Advantage Max (Thermo Finnigan) equipped with a Gemini C_{18} column (Phenomenex, 50 x 4.6 mm, 3μ), utilizing the following buffers: A: H_2O, B: acetonitrile and C: 1.0% $TFA_{(aq)}$. The deprotected pyrophosphates **1**–**3** were purified with ion-exchange chromatograpy (strong anion-exchange, 50 mM – 0.5 M ammonium acetate, 4 mL per minute) with detection at 280nM. The deprotected sugar nucleoside **18** was first run over Sephadex Q40 column with triethylammonium acetate (50 mM) buffer followed by purification on a Gilson GX-281 automated HPLC system, equipped with a preparative Gemini C_{18} column (Phenomenex, 150 x 21.20, 5 μ), using the buffers: A: triethylammonium acetate (50 $mM_{(aq)}$), B: acetonitrile (HPLC-grade). Purified products were lyophilised on a CHRIST ALPHA 2-4 LD_{PLUS} to remove water and buffer salts.

General experimental procedure for synthesis of sugar nucleotides. Phosphoramidite **7** (0.1 mmol, 1.0 eq) was coevaporated once in anhydrous MeCN (5.0 mL) and dissolved in anhydrous MeCN (1.5 mL) under an atmosphere of argon. Sugar phosphate **14a**, **14b**, or **14c** (0.12 mmol, 1.2 eq) and DCI (0.2 mmol, 2.0 eq) were coevaporated once in dry MeCN (5 mL) and then dissolved in anhydrous MeCN (2.0 mL). The latter solution was added to the phosphoramidite **7** at ambient temperature. The reaction mixture was stirred for 30 minutes after which ^{t}BuOOH (5.0 M in nonane) (0.4 mmol, 4.0 eq) was added. After 30 minutes of reaction time, DBU (0.5 mmol, 5.0 eq) was added and the reaction was stirred for an additional 30 minutes. Triethylamine (0.23 mL, 1.6 mmol, 16 eq) was then added followed by $(HF)_3{\cdot}Et_3N$ (0.8 mmol, 8.0 eq). Upon full desilylation, (about 4 hours) was aqueous ammonium hydroxide 25% (0.43 mL, 30 mmol, 30 eq) added and the reaction was stirred at ambient temperature over night. The mixture was then diluted with MilliQ (4 mL) and washed with DCM (4 mL). The organic layer was extracted once more with MilliQ (6 mL). The combined aqueous phases were concentrated *in vacuo* at no more than 30 °C. The crude product was then dissolved in MilliQ (4 mL) and centrifuged. The supernatant was applied to a strong anion exchange column and

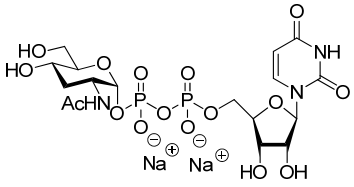
eluted with a gradient of ammonium acetate [0.05M (pH 7.0) - 0.5M (pH 7.1)] at 4 mL per minute. The fractions containing the product were collected and concentrated under reduced pressure. Repeated lyophilization (to remove residual ammonium acetate), followed by filtration over Amberlite Na^+-form, produced the desired sugar nucleotides.



Uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl diphosphate) disodium salt (1) As described in the general procedure for synthesis of sugar nucleotides, phosphoramidite **7** (53 mg, 0.1 mmol) was reacted with sugar phosphate **14a** (87 mg, 0.12 mmol, 1.2 eq) producing the title sugar nucleotide as a white hygroscopic solid (49 mg, 0.076 mmol, 76%). ^{1}H NMR (400 MHz, D_2O) δ: 8.02 (d, 1 H, *J* = 8.1 Hz, H-6), 6.05 (d, 1 H, *J* = 4.9 Hz, H-1′), 6.03 (d, 1 H, *J* = 8.1 Hz, H-5), 5.59 (dd, 1 H, *J* = 7.3, 3.3 Hz, H-1′′), 4.48 – 4.41 (m, 2 H, H-2′ and H-3′), 4.36 (m, 1 H, H-4′), 4.32 (m, 1 H, H-5a′), 4.26 (m, 1 H, H-5b′), 4.06 (ddd, 1 H, *J* = 10.4, 3.3, 3.1 Hz, H-2′′), 4.00 (ddd, 1 H, *J* = 9.7, 4.5, 2.4 Hz, H-5′′), 3.95 (dd, 1 H, *J* = 12.4, 2.4 Hz, H-6a′′), 3.92 – 3.84 (m, 2 H, H-6b′′ and H-3′′), 3.62 (t, 1 H, *J* = 9.7 Hz, H-4′′), 2.15 (s, 3 H, $CH_{3\text{-}NAc}$); ^{13}C NMR (101 MHz, D_2O) δ: 174.9 ($C{=}O_{NAc}$), 166.3 (C=O, C-4), 151.9 (C=O, C-2), 141.7 (C-6), 102.8 (C-5), 94.6 (d, *J* = 6.3 Hz, C-1′′), 88.6 (C-1′), 83.4 (d, *J* = 9.1 Hz, C-4′), 73.9 (C-3′), 73.1 (C-5′′), 71.1 (C-3′′), 69.8 (C-2′), 69.7 (C-4′′), 65.1 (d, *J* = 5.3 Hz, C-5′), 60.5 (C-6′′), 53.8 (d, *J* = 8.5 Hz, C-2′′), 22.2 ($CH_{3\text{-}NAc}$); ^{31}P NMR (162 MHz, D_2O) δ: -10.77 (d, 1 P, *J* = 21.1 Hz), -12.48 (d, 1 P, *J* = 21.1 Hz); HRMS Calcd. for $[C_{17}H_{27}N_3O_{17}P_2 + H]^+$: 608.0888, found 608.0889.

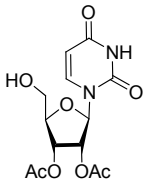


Uridine 5′-(2-acetamido-2-deoxy-3-*O*-methyl-α-D-glucopyranosyl diphosphate) disodium salt (2) As described in the general procedure for synthesis of sugar nucleotides, phosphoramidite **7** (53 mg, 0.1 mmol) was reacted with sugar phosphate **14b** (84 mg, 0.12 mmol, 1.2 eq) producing the title sugar nucleotide as a white solid (42 mg, 0.063 mmol, 63%). ^{1}H NMR (400 MHz, D_2O) δ: 8.00 (d, 1 H, *J* = 8.1 Hz, H-6), 6.04 – 5.97 (m, 2 H, H-1′ and H-5), 5.53 (dd, 1 H, *J* = 7.2, 3,3 Hz, H-1′′), 4.43 – 4.38 (m, 2 H, H-2′ and H-3′), 4.35 – 4.26 (m, 2 H, H-4′ and H-5_a′), 4.15 (ddd, 1 H, *J* = 11.7, 5.6, 3.1 Hz, H-5_b′), 4.09 (ddd, 1 H, *J* = 10.3, 3.3, 3.0 Hz, H-2′′), 3.98 (m, 1 H, H-5′′), 3.82 (dd, 1 H, *J* = 12.4, 2.2 Hz, H-6_a′′), 3.75 (dd, 1 H, *J* = 12.4, 4.3 Hz, H-6_b′′), 3.62 – 3.52 (m, 2 H, H-3′′ and H-4′′), 3.49 (s, 3 H, $CH_{3\text{-}OMe}$), 2.04 (s, 3 H, $CH_{3\text{-}NAc}$); ^{13}C NMR (101 MHz, D_2O) δ: 174.6 ($C{=}O_{NAc}$), 166.4 (C=O, C-4), 152.0 (C=O, C-2), 141.7 (C-6), 102.7 (C-5), 94.7 (d, *J* = 6.1 Hz, C-1′′), 88.5 (C-1′), 83.3 (d, *J* = 9.1 Hz, C-4′), 80.7 (C-3′′), 73.9 (C-2′), 73.1 (C-5′′), 69.7 (C-3′), 68.8 (C-4′′), 65.1 (d, *J* = 5.4 Hz, C-5), 60.3 (C-6′′), 59.6 ($CH_{3\text{-}OMe}$), 52.6 (d, *J* = 8.6 Hz, C-2′′), 22.2 ($CH_{3\text{-}NAc}$); ^{31}P NMR (162 MHz, D_2O) δ: -10.80 (d, 1 P, *J* = 21.1 Hz), -12.61 (d, 1 P, *J* = 21.1 Hz); HRMS Calcd for $[C_{18}H_{29}N_3O_{17}P_2{+}H]^+$: 622.1045, found 622.1044.

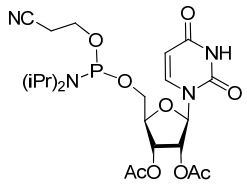


Uridine 5′-(2-acetamido-2,3-di-deoxy-α-D-glucopyranosyl diphosphate) disodium salt (3) As described in the general procedure for synthesis of sugar nucleotides, phosphoramidite **7** (53 mg, 0.1 mmol) was reacted with sugar phosphate **14c** (80 mg, 0.12 mmol, 1.2 eq) producing the title sugar nucleotide as a white solid (45 mg, 0.071 mmol, 71%). ^{1}H NMR (400 MHz, D_2O) δ: 8.01 (d, 1 H, *J* = 8.1 Hz, H-6), 6.04 – 5.98 (m, 2 H, H-1′ and H-5), 5.52 (dd, 1 H, *J* = 6,8, 3.0 Hz, H-1′′), 4.45 – 4.40 (m, 2 H, H-2′ and H-3′), 4.36 – 4.28 (m, 2 H, H-4′ and H-5_a′), 4.24 (ddd, 1 H, *J* = 11.7, 5.6, 2.9 Hz, H-5_b′), 4.10 (ddd, 1 H, *J* = 12.3, 7.0, 3.1 Hz, H-2′′), 3.92 – 3.81 (m, 3 H, H-5′′, H-6_a′′ and H-6_b′′), 3.80 – 3.73 (m, 1 H, H-4′′), 2.14 (m, 1 H, H-3_{eq}′′), 2.08 (s, 3 H, $CH_{3\text{-}NAc}$), 1.83 (dt, 1 H,

J = 12.3, 11.6 Hz, H-3$_{ax}$″); ^{13}C NMR (101 MHz, D_2O) δ: 173.9 (C=O_{NAc}), 166.4 (C=O, C-4), 151.9 (C=O, C-2), 141.7 (C-6), 102.7 (C-5), 93.3 (d, *J* = 6.3 Hz, C-1″), 88.7 (C-1′), 83.3 (d, *J* = 9.0 Hz, C-4′), 73.9 (C-3′), 73.6 (C-5″), 69.6 (C-2′), 65.0 (d, *J* = 5.4 Hz, C-5′), 63.7 (C-4″), 60.6 (C-6″), 48.0 (d, *J* = 8.8 Hz, C-2″), 31.7 (3-H″), 22.0 ($CH_{3\text{-}NAc}$); ^{31}P NMR (162 MHz, D_2O) δ: -10.72 (d, 1 P, *J* = 21.2 Hz), -12.20 (d, 1 P, *J* = 21.2 Hz); HRMS Calcd for $[C_{17}H_{27}N_3O_{16}P_2 + H]^+$: 592.0939, found 592.0937.

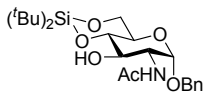


2′,3′-Di-*O*-acetyluridine (5) Uridine (12.2 g, 36.7 mmol, 1.0 eq) was suspended in dry DCE/pyridine 1:1 (300 mL) and triphenylmethyl chloride (13.6 g, 48.9 mmol, 1.3 eq) was added under argon. The mixture was heated to 50 °C and stirred over night. Upon completion of the reaction R_f: 0.64 (5% MeOH in EtOAc) was the reaction mixture cooled to room temperature and acetic anhydride (10.7 mL, 113 mmol, 3 mmol) was added via syringe. The reaction was then stirred at ambient temperature over night; R_f: 0.60 (EtOAc:DCM 1:1). The reaction mixture was concentrated *in vacuo* and redissolved in DCM (300 mL). The organics were then washed with 1 M HCl (300 mL), saturated bicarbonate (300mL) and brine (200mL). The aqeous layers were extracted using DCM (300 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*, giving a white foam. Acetic acid 80% aq. (300 mL) was added to the residue and the mixture was refluxed until TLC showed full conversion into a lower running product (3 hours) R_f: 0.15 (EtOAc:DCM 1:1). The reaction was cooled to room temperature and the solids were removed by suction filtration. The solids were washed with acetic acid/water 1:1 (100 mL). The filtrate was concentrated *in vacuo* and coevaporated with toluene (2 x 300 mL), producing a brownish oil. Purification by column chromatography (30–60% acetonitrile in DCM) produced the title compound as a white solid (11.7g, 35.6 mmol, 93% yield) over three steps. Compound characteristics were in agreement with those previously reported.[39]

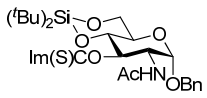


Uridine 2′,3′-Di-*O*-acetyl-5′-*O*-(*N*,*N*-diisopropylamino-*O*-[2-cyanoethyl])phosphoramidite (7). 2′,3′-Di-*O*-acetyl-uridine (2.0 g, 6.0 mmol, 1.0 eq) was coevaporated two times in anhydrous acetonitrile and was then dissolved in anhydrous DCM (18 mL). DIPEA (2.6 mL, 15 mmol, 3.0 eq) and 2-cyanoethyl-*N*,*N*-diisopropyl-amidochloridophosphite **6**[28] (1.4 g, 6.0 mmol, 1.0 eq) were consecutively added via a syringe under argon. The reaction was stirred at room temperature for one hour. The reaction mixture was then diluted with EtOAc (100mL), washed with sat. aq. bicarbonate (2x100 mL) and brine (80 mL). The aqueous layers were extracted with EtOAc (100 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (DCM:EtOAc 4:1 with 3% NEt_3) produced the title diastereoisomeric phosphoramidite as a colorless oil in a 3:2 ratio (3.1 g, 5.87 mmol, 98%) yield. This compound is stable at −20 °C over prolonged periods. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.25 (s, 1 H, $NH_{a/b}$), 7.86 (d, 0.6 H, *J* = 8.2 Hz, H-6$_a$), 7.78 (d, 0.4 H, *J* = 8.2 Hz, H-6$_b$), 6.32 (d, 0.6 H, *J* = 7.5 Hz, H-1$_a$′), 6.27 (d, 0.4 H, *J* = 12.4, 7.2 Hz, H-1$_b$′), 5.80 (d, 0.4 H, *J* = 6.0 Hz, H-5$_b$), 5.78 (d, 0.6 H, *J* = 6.1 Hz, H-5$_a$), 5.48 (dd, 0.6 H, *J* = 5.4, 1.6 Hz, H-3$_a$′), 5.40 (dd, 0.4 H, *J* = 5.5, 2.2 Hz, H-3$_b$′), 5.36 (dd, 0.6 H, *J* = 7.4, 5.5 Hz, H-2$_a$′), 5.31 (dd, 0.4 H, *J* = 7. 0, 5.6 Hz, H-2$_b$′), 4.30 (m, 1 H, C-4′$_{a/b}$), 4.01 – 3.90 (m, 1.8 H, C-5$_a$′ and *CH*$_2$CH$_2$C≡N$_a$), 3.90 – 3.76 (m, 1.2 H, C-5$_b$′ and *CH*$_2$CH$_2$C≡N$_b$), 3.71 – 3.57 (m, 2 H, $CH_{i\text{Pr-}a/b}$), 2.70 – 2.65 (m, 2 H, CH$_2$*CH*$_2$C≡N$_{a/b}$), 2.16 (s, 1.8 H, $CH_{3\text{-OAc-}a}$), 2.15 (s, 1.2 H, $CH_{3\text{-OAc-}b}$), 2.08 (s, 1.2 H, $CH_{3\text{-OAc-}b}$), 2.07 (s, 1.8 H, $CH_{3\text{-OAc-}a}$), 1.25 – 1.17 (m, 12 H, $CH_{3\text{-}i\text{Pr-}a/b}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.9 (C=$O_{OAc\text{-}a}$), 169.8 (C=$O_{OAc\text{-}b}$), 169.60 (C=$O_{OAc\text{-}a}$), 169.55 (C=$O_{OAc\text{-}b}$), 162.92, (C-4 x2), 150.7 (C-2$_a$), 150.5 (C-2$_b$), 139.6 (C-6 x2), 117.6 (C≡N$_a$), 117.4 (C≡N$_b$), 103.3 (C-4$_a$), 103.2 (C-4$_b$), 85.4 (C-1$_b$′), 84.9 (C-1$_a$′), 82.9 (d, *J* = 9.6 Hz, C-4$_a$′), 82.6 (d, *J* = 10.1 Hz, C-4$_b$′), 72.9 (C-2$_b$′), 72.6 (C-2$_a$′), 71.8 (C-3$_a$′), 71.5 (C-3$_b$′), 63.4 (d, *J* = 16.1 Hz, C-5$_b$′), 63.1 (d, *J* = 16.1 Hz, C-5$_a$′), 58.6 (d, *J* = 21.7 Hz, *CH*$_2$CH$_2$C≡N$_a$),

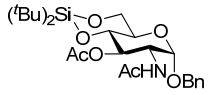
58.3 (d, *J* = 21.5 Hz, $CH_2CH_2C{\equiv}N_a$), 43.1 (d, *J* = 21.5 Hz, CH_{iPr}), 24.66, 24.64, 24.60, 24.57 ($CH_{3\text{-}iPr}$ x4), 20.66, 20.62, 20.42, 20.38, 20.36, 20.34, 20.30 ($CH_2CH_2C{\equiv}N$ and $CH_{3\text{-}OAc}$ x4); ^{31}P-NMR (162 MHz, $CDCl_3$) δ: 150.3, 149.2; IR (neat): 3064, 2969, 2936, 1749, 1693, 1458, 1377, 1236, 1050, 980, 811, 728 cm^{-1}. HRMS Calcd for $[C_{22}H_{33}N_4O_9P + Na]^+$: 551.1877, found: 551.1870.



Benzyl 2-acetamido-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-α-D-glucopyranoside (9) Di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (3.2 mL, 10 mmol, 1.0 eq.) was added dropwise to a cooled (−40 °C) solution of benzyl glycoside **8**[12] (3.3 g, 10.5 mmol, 1.05 eq) in anhydrous DMF (100 mL). The reaction mixture was stirred for 45 minutes at −40 °C, before anhydrous pyridine (2.4 mL, 30 mmol, 3.0 eq) was added. The reaction was allowed to reach room temperature and was then diluted with diethylether (300 mL), washed with sat. aq. $NaHCO_3$ (200 mL), H_2O (2 x 200 mL) and brine (150 mL). The aqueous phases were extracted with ether (300 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by column chromatography (20–30% acetone in petroleum ether) yielded the title compound as a white solid (4.26 g, 9.4 mmol, 94%). R_f = 0.21 (30% acetone in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 – 7.29 (m, 5 H, H_{arom}), 5.90 (d, 1 H, *J* = 8.8 Hz, NH), 4.90 (d, 1 H, *J* = 3.7 Hz, H-1), 4.68 (d, 1 H, *J* = 11.9 Hz, $CH_{2\text{-}OBn}$), 4.51 (d, 1 H, *J* = 11.9 Hz, $CH_{2\text{-}OBn}$), 4.14 (ddd, 1 H, *J* = 9.8, 8.8, 3.7 Hz, H-2), 4.01 (dd, 1H, *J* = 9.4, 3.6 Hz, H-6$_a$), 3.89 (t, 1 H, *J* = 9.5 Hz, H-6$_b$), 3.76 – 3.67 (m, 3 H, H-3, H-4 and H-5), 2.98 (bs, 1 H, OH), 1.97 (s, 3 H, $CH_{3\text{-}NAc}$), 1.05 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.97 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.7 ($C{=}O_{NAc}$), 137.1 ($C_{q\text{-}arom}$), 128.5, 128.1, 127.9 (C_{arom} x3), 97.2 (C-1), 77.9 (C-4), 72.5 (C-3), 70.1 ($CH_{2\text{-}OBn}$), 66.5 (C-5), 66.4 (C-6), 53.2 (C-2), 27.3, 26.9 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2 ($CH_{3\text{-}NAc}$), 22.6, 19.8 ($C_{q\text{-}tBu\text{-}Si}$ x2); Mp:79-82 °C, IR (neat): 3301, 2931, 2858, 1656, 1473, 1114, 1085, 1024, 825, 765 cm^{-1}; HRMS Calcd for $[C_{23}H_{37}NO_6Si + H]^+$: 452.2463, found 452.2461.

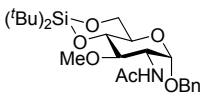


Benzyl 2-acetamido-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-3-*O*-thiocarbonylimidazole-α-D-glucopyranoside (10) Benzyl glycoside **9** (1.4 g, 3.0 mmol, 1.0 eq) was dissolved in anhydrous toluene (30 mL) and heated to 90 °C. Thiocarbonyldiimidazole (0.64 g, 3.6 mmol, 1.2 eq) was added and the reaction mixture was stirred at 90 °C over night. The reaction mixture was allowed to cool to room temperature and was then concentrated under reduced pressure. Purification by column chromatography (10–25% acetone in petroleum ether) gave benzyl-2-deoxy-2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-3-*O*-thiocarbonylimidazole-α-D-glucopyranoside as an off-white solid foam (1.7 g, 3.0 mmol, 99%). R_f = 0.33 (25% acetone in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.35 (s, 1 H, CH_{Im}), 7.60 (m, 1 H, CH_{Im}), 7.42 – 7.30 (m, 5 H, CH_{arom}), 7.02 (m, 1 H, CH_{Im}), 6.03 (d, 1 H, *J* = 9.6 Hz, NH), 6.00 (dd, 1 H, *J* = 10.9, 9.0 Hz, H-3), 4.93 (d, 1 H, *J* = 3.7 Hz, H-1), 4.73 (d, 1 H, *J* = 12.0 Hz, $CH_{2\text{-}OBn}$), 4.60 (d, 1 H, *J* = 12.0 Hz, $CH_{2\text{-}OBn}$), 4.56 (ddd, 1 H, *J* = 10.9, 9.6, 3.7 Hz, H-2), 4.10 (t, 1 H, *J* = 9.0 Hz, H-4), 4.01 (m, 1 H, H-6$_a$), 3.93 – 3.83 (m, 2 H, H-6$_6$ and H-5), 1.84 (s, 3 H, $CH_{3\text{-}NAc}$), 0.96 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.94 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 185.3 ($C{=}S_{Im}$), 169.8 ($C{=}O_{NAc}$), 137.2 (CH_{Im}), 136.6 ($C_{q\text{-}arom}$), 130.8 (CH_{Im}), 128.6, 128.3, 128.1 (CH_{arom} x3), 117.8 (CH_{Im}), 97.3 (C-1), 82.2 (C-3), 75.6 (C-4), 70.3 ($CH_{2\text{-}OBn}$), 66.9 (C-5), 66.2 (C-6), 51.3 (C-2), 27.2, 26.7 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.0 ($CH_{3\text{-}NAc}$), 22.5, 19.8 ($C_{q\text{-}tBu\text{-}Si}$ x2); IR (neat): 3249, 2933, 2860, 1662, 1384, 1226, 1087, 1045, 993, 825, 731 cm^{-1}. HRMS Calcd for $[C_{27}H_{39}N_3O_6SSi + H]^+$: 562.2402, found 562.2398.

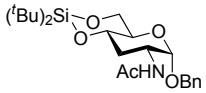


Benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-α-D-glucopyranoside (11a) Acetic anhydride (0.42 mL, 4.4 mmol, 2.0 eq) was added to a stirred solution of benzyl glycoside **9** (990 mg, 2.2 mmol, 1.0 eq) in pyridine (22 mL). The reaction was stirred until TLC showed full conversion to a higher running product. The reaction was then quenched with MeOH (0.5 mL) and diluted with EtOAc (40 mL). The organics

was washed with sat. aq $NaHCO_3$ (50mL) and brine (40 mL). The aqeous layers were extracted with EtOAc (40 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (10–25% acetone in petroleum ether) gave the title compound as a white solid (1.08 g, 2.2 mmol, 99%). R_f = 0.26 (20% acetone in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 – 7.28 (m, 5 H, H_{arom}), 5.74 (d, 1 H, *J* = 9.5 Hz, NH), 5.12 (dd, 1 H, *J* = 10.7, 8.9 Hz, H-3), 4.87 (d, 1 H, *J* = 3.7 Hz, H-1), 4.69 (d, 1 H, *J* = 11.9 Hz, $CH_{2\text{-}OBn}$), 4.54 (d, 1 H, *J* = 11.9 Hz, $CH_{2\text{-}OBn}$), 4.24 (ddd, 1 H, *J* = 10.7, 9.7, 3.7 Hz, H-2), 3.98 (dd, 1 H, *J* = 9.3, 4.3 Hz, H-6a), 3.91 – 3.82 (m, 2 H, H-6_a and H-5), 3.78 (m, 1 H, H-5), 2.07 (s, 3 H, $CH_{3\text{-}OAc}$), 1.90 (s, 3 H, $CH_{3\text{-}NAc}$), 1.02 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.95 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.4 ($C{=}O_{NAc}$), 169.9 ($C{=}O_{OAc}$), 136.9 ($C_{q\text{-}arom}$), 128.6, 128.2, 128.1 (C_{arom} x3), 97.3 (C-1), 75.1 (C-4), 73.1 (C-3), 70.3 ($CH_{2\text{-}OBn}$), 66.9 (C-5), 66.5 (C-6), 51.8 (C-2), 27.3, 26.8 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.1 ($CH_{3\text{-}NAc}$), 22.6 ($C_{q\text{-}tBu\text{-}Si}$), 20.7 ($CH_{3\text{-}OAc}$), 19.9 ($C_{q\text{-}tBu\text{-}Si}$); Mp:104-108 °C, IR (neat): 2935, 2861, 1752, 1656, 1474, 1231, 1089, 827, 733 cm^{-1}; HRMS Calcd for $[C_{18}H_{31}NO_6Si + H]^+$: 386.1999, found 386.1994.

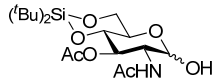


Benzyl 2-acetamido-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-3-*O*-methyl-*α*-D-glucopyranoside (11b). Benzyl glycoside **9** (1.4 g, 3.0 mmol, 1.0 eq) was dissolved in anhydrous DCM (15 mL) under argon and cooled to −40 °C. $BF_3{\cdot}Et_2O$ (0.74 mL, 6.0 mmol, 2.0 eq) was added followed by trimethylsilyldiazomethane (2 M in hexanes, 3.0 mL, 6.0 mmol, 2.0 eq). The reaction mixture was stirred for 45 minutes at −40 °C and quenched with acetic acid (1 mL). The mixture was poured into EtOAc (100 mL) and washed with sat. aq. $NaHCO_3$ (100 mL) and brine (80 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (10–20% acetone in petroleum ether) produced the title compound as a white solid (0.87 g, 1.87 mmol, 62%). R_f = 0.45 (25% acetone in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 – 7.30 (m, 5 H, H_{arom}), 5.64 (d, 1 H, *J* = 9.1 Hz, NH), 4.90 (d, 1 H, *J* = 3.7 Hz, H-1), 4.67 (d, 1 H, *J* = 11.8 Hz, $CH_{2\text{-}OBn}$), 4.50 (d, 1 H, *J* = 11.8 Hz, $CH_{2\text{-}OBn}$), 4.12 (ddd, 1 H, *J* = 10.5, 9.1, 3.7 Hz, H-2), 3.98 (dd, 1 H, *J* = 9.8, 4.8 Hz, H-6_a), 3.91 (dd, 1 H, *J* = 9.5, 8.7 Hz, H-4), 3.85 (dd, 1 H, *J* = 10.0, 9.8 Hz, H-6_b), 3.75 (ddd, 1 H, *J* = 10.0, 9.5, 4.8 Hz, H-5), 3.59 (s, 3 H, $CH_{3\text{-}OMe}$), 3.34 (dd, 1 H, *J* = 10.5, 8.7 Hz, H-3), 1.95 (s, 3 H, $CH_{3\text{-}NAc}$), 1.05 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.99 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.9 ($C{=}O_{NAc}$), 137.2 ($C_{q\text{-}arom}$), 128.5, 128.1, 128.0 (CH_{arom} x3), 97.5 (C-1), 80.7 (C-3), 78.4 (C-4), 70.1 ($CH_{2\text{-}OBn}$), 66.8 (C-5), 66.6 (C-6), 60.1 ($CH_{3\text{-}OMe}$), 51.8 (C-2), 27.3, 26.9 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.3 ($CH_{3\text{-}NAc}$), 22.5, 19.8 ($C_{q\text{-}tBu\text{-}Si}$ x2); Mp:146-149 °C; IR (neat): 3267, 2858, 1651, 1471, 1128, 1080, 1041, 825, 765 cm^{-1}; HRMS Calcd for $[C_{24}H_{39}NO_6Si + Na]^+$: 488.2439, found 488.2432.

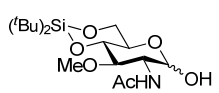


Benzyl 2-acetamido-2,3-di-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-*α*-D-glucopyranoside (11c) To a heated solution (95 °C) of tributyltinhydride (1.2 mL, 4.4 mmol, 1.5 eq) in anhydrous toluene (20 mL) was added a solution of benzyl 2-deoxy-2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-3-*O*-thiocarbonylimidazole-*α*-D-glucopyranose **10** (1.7 g, 2.9 mmol, 1.0 eq) in anhydrous toluene (10 mL) and AIBN (48 mg, 0.29 mmol, 0.1 eq). After 4 hours, TLC analysis (EtOAc/Toluene, 6:4) showed full conversion to a higher running product. The mixture was allowed to cool to room temperature, filtered over Celite and concentrated under reduced pressure. Purification by column chromatography (15–35% EtOAc in petroleum ether) gave the title deoxysugar as a white solid (1.20 g, 2.75 mmol, 94%). R_f = 0.49 (25% acetone in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 – 7.29 (m, 5 H, H_{arom}), 5.73 (d, 1 H, *J* = 9.2 Hz, NH), 4.75 (d, 1 H, *J* = 3.6 Hz, H-1), 4.72 (d, 1 H, *J* = 11.8 Hz, $CH_{2\text{-}OBn}$), 4.52 (d, 1 H, *J* = 11.8 Hz, $CH_{2\text{-}OBn}$), 4.20 (dddd, 1 H, *J* = 12.6, 9.2, 4.5, 3.6 Hz, H-2), 3.96 (dd, 1 H, *J* = 9.9, 4.9 Hz, H-6_a), 3.90 – 3.82 (m, 1 H, H-4), 3.80 (t, 1 H, *J* = 9.9 Hz, H-6_b), 3.65 (ddd, 1 H, *J* = 10.4, 9.9, 4.9 Hz, H-5), 2.17 (ddd, 1 H, *J* = 11.8, 4.7, 4.3 Hz, H-3_{eq}), 1.92 (s, 3 H, $CH_{3\text{-}NAc}$), 1.68 (ddd, 1 H, *J* = 12.6, 12.5, 11.8 Hz, H-3_{ax}),

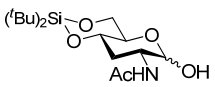
1.03 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.97 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.2 ($C{=}O_{NAc}$), 137.4 ($C_{q\text{-}arom}$), 128.5, 128.0, 128.0 (CH_{arom} x3), 96.0 (C-1), 72.0 (C-4), 69.6 ($CH_{2\text{-}OBn}$), 68.2 (C-5), 66.8 (C-6), 47.1 (C-2), 34.0 (C-3), 27.3, 27.0 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.2 ($CH_{3\text{-}NAc}$), 22.5, 19.8 ($C_{q\text{-}tBu\text{-}Si}$ x2); Mp:112-116 °C; IR (neat): 3261, 2935, 2860, 1651, 1558, 1070, 1010, 827, 736 cm^{-1}; HRMS Calcd for $[C_{23}H_{37}NO_5Si + H]^+$: 436.2514, found 436.2512.



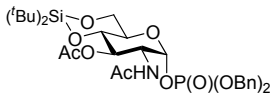
2-Acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-α/β-D-glucopyranose (12a). A stirred solution of benzyl glycoside **(11a)** (0.72 g, 1.5 mmol, 1.0 eq) in THF (15 mL), was flushed with argon. A catalytic amount of $Pd(OH)_2$ on charcoal 20 mol% (100 mg, 0.15 mmol, 0.1 eq) and AcOH (0.15 mL) was added. The mixure was then purged with hydrogen gas and kept under a hydrogen atmosphere over night. The reaction mixture was filtered over a Whatmann paper and the filter was carefully rinsed with methanol (20 mL). Concentration *in vacuo* and purification by column chromatography (20–40% acetone in petroleum ether) produced the title compound in a mixture of anomers (5:1 *α/β*), as a white solid (0.55 g, 1.36 mmol, 93%). R_f = 0.26 (20% acetone in DCM); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.40 (d, 0.2 H, *J* = 6.9 Hz, NH_β), 6.02 (d, 1 H, *J* = 9.3 Hz, NH_α), 5.19 (d, 1 H, *J* = 5.6 Hz, H-1_α), 5.17 (dd, 1 H, *J* = 10.8, 9.2 Hz, H-3_α) 4.88 (dd, 0.2 H, *J* = 10.7, 9.0 Hz, H-3_b), 4.63 (d, 0.2 H, *J* = 8.3 Hz, H-1_b), 4.24 (dd, 0.2 H, *J* = 10.4, 5.2 Hz, H-$6_{a\text{-}\beta}$), 4.18 (ddd, 1 H, *J* = 10.7, 9.4, 3.6 Hz, H-2_α), 4.12 – 4.05 (m, 1.2 H, H-$6_{b\text{-}\beta}$ and H-$6_{a\text{-}\alpha}$), 4.03 (dd, 1 H, *J* = 9.8, 4.8 Hz, H-$6_{b\text{-}\alpha}$), 3.97 – 3.84 (m, 2.4 H, H-2_β, H-4_β, H-4_α and H-5_α), 3.46 (td, 0.2 H, *J* = 9.9, 5.0 Hz, H-5_β), 2.15 (s, 0.6 H, $CH_{3\text{-}OAc\text{-}\beta}$), 2.09 (s, 3 H, $CH_{3\text{-}OAc\text{-}\alpha}$), 2.02 (s, 0.6 H, $CH_{3\text{-}NAc\text{-}\beta}$), 1.97 (s, 3 H, $CH_{3\text{-}NAc\text{-}\alpha}$), 1.04 (s, 1.8 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 1.03 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 0.97 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 0.97 (s, 1.8 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.7 ($C{=}O_{NAc\text{-}\beta}$), 172.7 ($C{=}O_{OAc\text{-}\beta}$), 171.6 ($C{=}O_{NAc\text{-}\alpha}$), 170.6 ($C{=}O_{NAc\text{-}\alpha}$), 98.2 (C-$1_\beta$), 91.8 (C-$1_\alpha$), 75.2 (C-$4_\alpha$), 74.5 (C-$3_\beta$), 72.8 (C-$3_\alpha$), 70.6 (C-$5_\beta$), 66.7 (C-$6_\alpha$ and C-4 $_\beta$), 66.5 (C-5_α), 66.2 (C-6_β), 57.2 (C-2_β), 52.3 (C-2_α), 27.33 ($CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 27.30 ($CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 26.9 ($CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 26.8 ($CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 23.1 ($CH_{3\text{-}NAc\text{-}\alpha}$), 23.0 ($CH_{3\text{-}NAc\text{-}\beta}$), 22.63 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha}$), 22.61 ($C_{q\text{-}tBu\text{-}Si\text{-}\beta}$), 20.8 ($CH_{3\text{-}OAc\text{-}\alpha}$), 20.7 ($CH_{3\text{-}OAc\text{-}\beta}$), 19.9 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha}$ and $C_{q\text{-}tBu\text{-}Si\text{-}\beta}$); Mp:162-165 °C; IR (neat): 3310, 2935, 2861, 1733, 1659, 1227, 1054, 825, 732 cm^{-1}; HRMS Calcd for $[C_{18}H_{33}NO_7Si + H]^+$: 404.2105, found 404.2099.



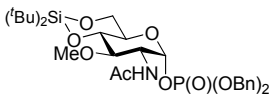
2-acetamido-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-3-*O*-Methyl-*α/β*-D-glucopyranose (12b). Benzyl glycoside **11b** (0.70 g, 1.5 mmol, 1.0 eq) was dissolved in dioxane:MeOH (3:1, 15 mL) and flushed with argon. A catalytic amount of $Pd(OH)_2$ on charcoal 20 mol% (100 mg, 0.15 mmol, 0.1 eq) and AcOH (0.1 mL) was added. The mixture was purged with hydrogen followed by stirring under a hydrogen atmosphere for 16 hours. The reaction mixture was filtered over Whatmann paper and the filter was then rinsed with MeOH (30 mL). Concentration under reduced pressure and purification by column chromatography (20–30% acetone in DCM) produced the title compound as a mixture of anomers (5:1 α/β), as a white solid (0.55 g, 1.46 mmol, 97 %). R_f = 0.31 (20% acetone in DCM); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.13 (d, 0.2 H, *J* = 5.7 Hz, NH_β), 5.95 (d, 1 H, *J* = 8.7 Hz, NH_α), 5.18 (d, 1 H, *J* = 3.3 Hz, H-1_α), 4.78 – 4.61 (m, 1.4 H, H-1_β, OH_β and OH_α), 4.19 (dd, 0.2 H, *J* = 10.3, 5.0 Hz, H-$6_{a\text{-}\beta}$), 4.07 (dd, 1 H, *J* = 9.6, 4.6 Hz, H-$6_{a\text{-}\alpha}$), 4.02 – 3.77 (m, 4.4 H, H-2_α, H-5_α, H-4_α, H-4_β, H-$6_{b\text{-}\alpha}$, and H-$6_{b\text{-}\beta}$), 3.64 (s, 0.6 H, $CH_{3\text{-}OMe\text{-}\beta}$), 3.63 – 3.59 (m, 3,2 H, $CH_{3\text{-}OMe\text{-}\alpha}$ and H-2_β), 3.46 – 3.38 (m, 1.2 H, H-3_α and H-5_β), 3.31 (dd, 0.2 H, *J* = 10.4, 8.4 Hz, H-3_β), 2.08 (s, 0.6 H, $CH_{3\text{-}NAc\text{-}\beta}$), 2.02 (s, 3 H, $CH_{3\text{-}NAc\text{-}\alpha}$), 1.06 (s, 1.8 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 1.05 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 1.01 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 1.00 (s, 1.8 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 174.1 ($C{=}O_{NAc\text{-}\beta}$), 170.9 ($C{=}O_{NAc\text{-}\alpha}$), 97.8 (C-$1_\beta$), 91.9 (C-$1_\alpha$), 81.5 (C-$3_\beta$), 80.2 (C-$3_\alpha$), 78.6 (C-$4_\alpha$), 78.0 (C-$4_\beta$), 70.4 (C-$5_\beta$), 66.8 (C-$6_\alpha$), 66.4 (C-$5_\alpha$), 66.3 (C-$6_\beta$), 60.2 ($CH_{3\text{-}OMe\text{-}\alpha}$), 59.8 ($CH_{3\text{-}OMe\text{-}\beta}$), 57.3 (C-$2_\beta$), 52.8 (C-$2_\alpha$), 27.41 ($CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 27.39 ($CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 27.02 ($CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 27.00 ($CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 23.3 ($CH_{3\text{-}NAc\text{-}\alpha}$), 23.1 ($CH_{3\text{-}NAc\text{-}\beta}$), 22.63 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha}$), 22.60 ($C_{q\text{-}tBu\text{-}Si\text{-}\beta}$), 19.90 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha}$), 19.89 ($C_{q\text{-}tBu\text{-}Si\text{-}\beta}$); Mp:224-227 °C; IR (neat): 3276, 2931, 2858, 1647, 1552, 1365, 1083, 825, 767 cm^{-1}; HRMS Calcd for $[C_{17}H_{33}NO_6Si + H]^+$: 376.2150, found 376.2151.



2-Acetamido-2,3-di-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-*α*/*β*-D-glucopyranose (12c). Benzyl glycoside **11c** (0.90 g, 2.1 mmol, 1.0 eq) was dissolved in dioxane:MeOH (3:1, 20 mL) and purged with argon. A catalytic amount of $Pd(OH)_2$ on charcoal 20 mol% (145 mg, 0.21 mmol, 0.1 eq) and of AcOH (0.15 mL) was added. The mixture was purged with hydrogen, followed by stirring under a hydrogen atmosphere for 16 hours. The reaction mixture was filtered over a Whatmann paper and the filter was rinsed with methanol (20 mL). Concentration *in vacuo* and purification by column chromatography (20–30% acetone in DCM) produced the title compound as a mixture of anomers (5:1 α/β), as an amorphous solid (0.70 g, 2.03 mmol, 98 %). R_f = 0.29 (20% acetone in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.78 (d, 1 H, *J* = 9.1 Hz, NH_α), 5.74 (d, 0.2 H, *J* = 8.0 Hz, NH_β), 5.37 (d, 0.2 H, *J* = 6.3 Hz, OH_β), 5.07 (dd, 1 H, *J* = 3.8, 2.5 Hz, H-1$_\alpha$), 4.51 (dd, 0.2 H, *J* = 8.2, 5.9 Hz, H-1$_\beta$), 4.22 – 4.12 (m, 1.2 H, H-2$_\alpha$, H-6$_{a\text{-}\beta}$), 4.04 (dd, 1 H, *J* = 8.4, 2.9 Hz, H-6$_\alpha$), 3.95 – 3.77 (m, 3.6 H, H-5$_\alpha$, H-4$_\beta$, H-4$_\alpha$, H-6$_{b\text{-}\alpha}$, H-6$_{b\text{-}\beta}$ and H-2$_\beta$), 3.61 (bs, 1 H, OH_α), 3.39 (m, 0.2 H, H-5$_\beta$), 2.33 (ddd, 0.2 H, *J* = 12.2, 4.7, 4.4 Hz, H-3$_{eq\text{-}\beta}$), 2.18 (ddd, 1 H, *J* = 11.9, 4.4, 4.0 Hz, H-3$_{eq\text{-}\alpha}$), 2.05 (s, 0.6 H, $CH_{3\text{-}NAc\text{-}\beta}$), 1.99 (s, 3 H, $CH_{3\text{-}NAc\text{-}\alpha}$), 1.70 (m, 1 H, H-3$_{ax\text{-}\alpha}$), 1.53 (ddd, 0.2 H, *J* =12.6, 12.2, 10.7 Hz, H-3$_{ax\text{-}\beta}$) 1.04 (s, 10.8 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$ and $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 0.99 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 0.98 (s, 1.8 H, $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.9 ($C{=}O_{NAc\text{-}\beta}$), 169.6 ($C{=}O_{NAc\text{-}\alpha}$), 99.9 (C-1$_\beta$), 90.6(C-1$_\alpha$), 74.7 (C-5$_\beta$), 72.2 (C-5$_\alpha$), 71.8 (C-4$_\beta$), 67.9 (C-4$_\alpha$), 67.0 (C-6$_\alpha$), 66.6 (C-6$_\beta$), 51.8 (C-2$_\beta$), 47.5 (C-2$_\alpha$), 37.1 (C-3$_\beta$), 33.3 (C-3$_\alpha$), 27.43 ($CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 27.40 ($CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 27.0 ($CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 26.9 ($CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$), 23.3 ($CH_{3\text{-}NAc\text{-}\alpha}$), 23.0 ($CH_{3\text{-}NAc\text{-}\beta}$), 22.65 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha}$), 22.63 ($C_{q\text{-}tBu\text{-}Si\text{-}\beta}$), 19.9 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha}$ and $C_{q\text{-}tBu\text{-}Si\text{-}\beta}$); Mp:189-192 °C; IR (neat): 3304, 2935, 2861, 1652, 1474, 1101, 826, 734 cm^{-1}; HRMS Calcd for $[C16H31NO_5Si + H]^+$: 346.2044, found: 346.2045.

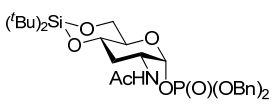


(2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-*α*-D-glucopyranosyl)-dibenzylphosphate (13a). Hemiacetal **12a** (0.39 g, 0.97 mmol, 1.0 eq) was dissolved in anhydrous THF (10 mL) and cooled to −78 °C. LDA (1.8 M in THF) (0.58 mL, 1.02 mmol, 1.05 eq) was added and the reaction was stirred for 5 minutes before tetrabenzyl pyrophosphate (0.63 g, 1.2 mmol, 1.2 eq) dissolved in dry THF (4 mL) was added. The reaction was left to reach room temperature before it was diluted with EtOAc (30 mL). The organics was washed with sat. aq. $NaHCO_3$ (50 mL) and brine (40 mL). The aqueous layers were extracted using EtOAc (50 mL). The combined organic layers were dried with Na_2SO_4, filtrated and concentrated under reduced preassure. Purification by column chromatography (DCM:EtOAc 1:1) gave the title compound as a clear oil (0.63 g, 0.95 mmol, 98%). R_f = 0.31 (50% EtOAc in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 – 7.30 (m, 10 H, H_{arom}), 5.65 – 5.58 (m, 2 H, H-1 and NH), 5.11 – 5.01 (m, 5 H, C-3 and $CH_{2\text{-}Bn}$ x2), 4.29 (dddd, 1 H, *J* = 10.9, 9.3, 3.3, 3.1 Hz, H-2), 4.00 – 3.85 (m, 3 H, H-4, H-5, H-6$_a$), 3.81 (m, 1 H, H-6$_b$), 2.07 (s, 3 H, $CH_{3\text{-}OAc}$), 1.71 (s, 3 H, $CH_{3\text{-}NAc}$), 1.02 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.94 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.2 ($C{=}O_{NAc}$), 170.1 ($C{=}O_{OAc}$), 135.3 (d, *J* = 6.5 Hz, $C_{q\text{-}arom}$), 135.2 (d, *J* = 7.0 Hz, $C_{q\text{-}arom}$), 128.9 (x2), 128.8, 128.7, 128.0, 127.9 (CH_{arom} x6), 96.5 (d, *J* = 6.5 Hz, C-1), 74.4 (C-4), 71.9 (C-3), 69.8 (d, *J* = 5.9 Hz, $CH_{2\text{-}OBn}$), 69.7 (d, *J* = 5.7 Hz, $CH_{2\text{-}OBn}$), 68.4 (C-5), 66.1 (C-6), 51.6 (d, *J* = 8.0 Hz, C-2), 27.2, 26.8 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 22.8 ($CH_{3\text{-}NAc}$), 22.6 ($C_{q\text{-}tBu\text{-}Si}$), 20.7 ($CH_{3\text{-}OAc}$), 19.9 ($C_{q\text{-}tBu\text{-}Si}$); ^{31}P NMR (162 MHz, $CDCl_3$) δ: -2.17 (s, 1 P); IR (neat): 2934, 2860, 1751, 1686, 1230, 957, 827 cm^{-1}; HRMS Calcd for $[C_{32}H_{46}NO_{10}PSi + H]^+$: 664.2707, found: 664.2708.

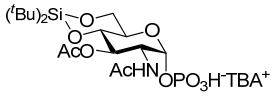


(2-acetamido-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-3-*O*-Methyl-*α*-D-glucopyranosyl)-dibenzylphosphate (13b). Hemiacetal **12b** (0.38 g, 1.00 mmol, 1.0 eq) was dissolved in dry THF (10 mL) and cooled to −78 °C. LDA (1.8 M in THF) (0.58 mL, 1.05 mmol, 1.05 eq) was added and the reaction was stirred for 5 minutes before addition of tetrabenzyl pyrophosphate (0.59g, 1.1 mmol, 1.1 eq) dissolved in

anhydrous THF (4 mL). The reaction was left to reach room temperature and was then diluted with EtOAc (40 mL). The organics was washed with sat. aq. $NaHCO_3$ (50 mL) and brine (40 mL). The aqueous layers were extracted using EtOAc (50 mL) and the combined organic layers were dried (Na_2SO_4), filtrated and concentrated under reduced preassure. Purification by column chromatography (15–30 % acetone in petroleum ether, 3 % NEt_3) afforded the title compound as a clear oil (0.53 g, 0.84 mmol, 94%). R_f = 0.35 (20 % acetone in petroleum ether, 3 % NEt_3); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.45 – 7.34 (m, 10 H, H_{arom}), 5.64 (dd, 1 H, *J* = 5.5, 3.4 Hz, H-1), 5.41 (d, 1 H, *J* = 8.9 Hz, NH), 5.15 – 5.03 (m, 4 H, $CH_{2\text{-}OBn}$ x2), 4.15 (dddd, 1 H, *J* = 10.7, 8.9, 3.4, 3.3 Hz, H-2), 3.97 (dd, 1 H, *J* = 8.9, 4.0 Hz, H-6_a), 3.92 – 3.85 (m, 2 H, H-4 and H-5), 3.87 – 3.79 (dd, 1 H, *J* = 9.5, 8.9 Hz, H-6_b), 3.56 (s, 3 H, $CH_{3\text{-}OMe}$), 3.18 (dd, 1 H, *J* = 10.6, 8.1 Hz, H-3), 1.84 (s, 3 H, $CH_{3\text{-}OAe}$), 1.04 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.98 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1 ($C{=}O_{NAc}$), 135.6 (d, *J* = 6.1 Hz, $C_{q\text{-}arom}$), 135.4 (d, *J* = 6.7 Hz, $C_{q\text{-}arom}$), 128.9, 128.7 (x3), 128.0, 127.9 (CH_{arom} x6), 97.1 (d, *J* = 6.8 Hz, C-1), 79.7 (C-3), 77.8 (C-4), 69.8 (d, *J* = 5.4 Hz, $CH_{2\text{-}OBn}$ x2), 68.4 (C-5), 66.2 (C-6), 60.3 ($CH_{3\text{-}OMe}$), 51.8 (d, *J* = 7.8 Hz, C-2), 27.3, 27.0 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 23.0 ($CH_{3\text{-}NAc}$), 22.6, 19.9 ($C_{q\text{-}tBu\text{-}Si}$ x2). ^{31}P NMR (162 MHz, CDCl3) δ: -1.85 (s, 1 P); IR (neat): 3291, 2935, 2860, 1652, 1270, 943, 826, 734 cm^{-1}; HRMS Calcd for $[C_{31}H_{46}NO_9PSi + H]^+$: 636.2758, found: 636.2756.

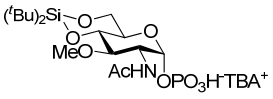


(2-acetamido-2,3-di-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-*α*-D-glucopyranosyl)-dibenzylphosphate (13c). Hemiacetal **12c** (0.35 g, 1.0 mmol, 1.0 eq) was dissolved in anhydrous THF (10 mL) and cooled to −78 °C. LDA (1.8 M in THF) (0.58 mL, 1.05 mmol, 1.05 eq) was added and the reaction was stirred for 5 minutes before addition of tetrabenzyl pyrophosphate (0.59 g, 1.10 mmol, 1.1 eq) dissolved in dry THF (4 mL). The reaction was left to reach room temperature and was then diluted with EtOAc (40 mL). The organics was washed with sat. aq. $NaHCO_3$ (50 mL) and brine (40 mL). The aqueous layers were extracted using EtOAc (50 mL) and the combined organic layers were dried (Na_2SO_4), filtrated and concentrated under reduced preassure. Purification by column chromatography (0–10% acetone, 40% DCM in petroleum ether, 3% NEt_3) afforded the title compound as a white solid (0.51 g, 0.84 mmol, 84%). R_f = 0.31 (20 % acetone in petroleum ether, 3 % NEt_3); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37 – 7.40 (m, 10 H, H_{arom}), 5.56 (d, 1 H, *J* = 9.1 Hz, NH), 5.50 (dd, 1 H, *J* = 5.4, 3.2 Hz, H-1), 5.14 – 5.02 (m, 4 H, $CH_{2\text{-}OBn}$ x2), 4.22 (m, 1 H, H-2), 3.94 (dd, 1 H, *J* = 15.8, 10.8 Hz, H-6_a), 3.86 (m, 1 H, H-4), 3.80 – 3.71 (m, 2 H, H-5 and H-6_b), 2.13 (ddd, 1 H, *J* = 12.0, 4.5, 4.2 Hz, H-3_{eq}), 1.77 (s, 3 H, $CH_{3\text{-}NAc}$), 1.54 (ddd, 1 H, *J* = 12.4, 12.0, 10.9 Hz, H-3_{ax}), 1.02 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.96 (s, 9 H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.5 ($C{=}O_{NAc}$), 135.6 (d, *J* = 6.3 Hz, $C_{q\text{-}arom}$), 135.3 (d, *J* = 6.9 Hz, $C_{q\text{-}arom}$), 128.9 (x2), 128.74, 128.73, 128.0, 127.9 (CH_{arom} x6), 95.9 (d, *J* = 6.6 Hz, C-1), 71.3 (C-4), 69.78 (d, *J* = 5.7 Hz, $CH_{2\text{-}OBn}$), 69.74 (d, *J* = 5.6 Hz, $CH_{2\text{-}OBn}$), 69.67 (C-5), 66.5 (C-6), 47.1 (d, *J* = 7.6 Hz, C-2), 33.1 (C-3), 27.4, 27.0 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 22.9 ($CH_{3\text{-}NAc}$), 22.6, 19.9 ($C_{q\text{-}tBu\text{-}Si}$ x2); ^{31}P-NMR (162 MHz, $CDCl_3$) δ: -1.92 (s, 1 P); Mp: 141-144 °C; IR (neat): 3314, 2931, 2858, 1253, 1107, 943 cm^{-1}; HRMS Calcd for $[C_{30}H_{44}NO_8PSi + H]^+$: 606.2647, found: 606.2607.

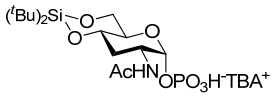


2-Acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-*α*-D-glucopyranosylphosphate, mono-tetrabutylammonium salt (14a). Dibenzylphosphotriester **13a** (0.27 g, 0.4 mmol, 1.0 eq) was dissolved in methanol (8 mL) and purged with argon. Palladium on charcoal (Pd/C, 10 mol%) (42 mg, 0.08 mmol, 0.1 eq) was added and the reaction was purged with hydrogen gas and then stirred under a hydrogen atmosphere for three hours at ambient temperature. The mixture was then filtered over Whatmann paper and the filter was rinsed with MeOH (20 mL). Concentration of the organics under reduced pressure followed by addition of 40% aq. solution of tetrabutylammonium hydroxide (0.26 mL, 0.40 mmol, 1.0 eq. based on dry sugar phosphate) and subsequent lyophilization, afforded the title compound as a white hygroscopic compound

(0.29 g, 0.39 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (d, 1 H, *J* = 8.9 Hz, NH), 7.24 (bs, 1 H, POH), 5.48 (dd, 1 H, *J* = 7.8, 2.8 Hz, H-1), 5.21 (t, 1 H, *J* = 9.8 Hz, H-3), 4.24 (ddd, 1 H, *J* = 9.6, 8.9, 3.5 Hz, H-2), 4.16 (dd, 1 H, *J* = 9.4, 5.0 Hz, H-6_a), 4.09 (dd, 1 H, *J* = 9.5, 5.0 Hz, H-6_b), 3.89 (t, 1 H, *J* = 9.2 Hz, H-4), 3.80 (t, 1 H, *J* = 9.4 Hz, H-5), 3.37 – 3.24 (m, 8 H, $CH_{2\text{-TBA}}$), 2.03 (s, 3 H, $CH_{3\text{-OAc}}$), 1.98 (s, 3 H, $CH_{3\text{-NAc}}$), 1.74 – 1.57 (m, 8 H, $CH_{2\text{-TBA}}$), 1.50 – 1.38 (m, 8 H, $CH_{2\text{-TBA}}$), 1.02 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 1.00 (t, 12 H, *J* = 7.5 Hz, $CH_{3\text{-TBA}}$), 0.95 (s, 9 H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.2 ($C{=}O_{NAc}$), 170.6 ($C{=}O_{NOc}$), 93.6 (d, *J* = 4.0 Hz, C-1), 75.5 (C-4), 73.6 (C-3), 67.2 (C-5), 67.0 (C-6), 58.7 ($CH_{2\text{-TBA}}$), 52.43 (d, *J* = 4.9 Hz, C-2), 27.4, 26.9 ($CH_{3\text{-tBu-Si}}$ x2), 23.9 ($CH_{2\text{-TBA}}$), 23.2 ($CH_{3\text{-NAc}}$), 22.6 ($C_{q\text{-tBu-Si}}$), 20.8 ($CH_{3\text{-OAc}}$), 19.8 ($C_{q\text{-tBu-Si}}$), 19.6 ($CH_{2\text{-TBA}}$), 13.6 ($CH_{3\text{-TBA}}$); ^{31}P NMR (162 MHz, $CDCl_3$) δ: -0.18 (s, 1 P); IR (neat): 3261, 2963, 2861, 1740, 1669, 1234, 1087, 826 cm^{-1}; HRMS Calcd for $[C_{18}H_{34}NO_{10}PSi + Na]^+$: 506.1587, found: 506.1581.

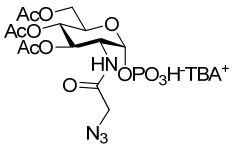


2-Acetamido-2-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-3-*O*-methyl-*α*-D-glucopyranosylphosphate, mono-tetrabutylammonium salt (14b). Dibenzylphosphotriester **13b** (0.54 g, 0.84 mmol, 1.0 eq) was dissolved in methanol (17 mL) and purged with argon. Palladium on charcoal (Pd/C, 10 mol%) (90 mg, 0.08 mmol, 0.1 eq) was added and the reaction was purged with hydrogen gas and then stirred under a hydrogen atmosphere for three hours at ambient temperature. The mixture was filtered over a Whatmann paper and the filter was rinsed with methanol (20 mL). The organics were concentrated under reduced pressure and 40% aq. solution of tetrabutylammonium hydroxide (0.55 mL, 0.84 mmol, 1.0 eq. based on dry sugar phosphate) was added. Subsequent lyophilization, afforded the title compound as white hygroscopic solids (0.58 g, 0.13 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.42 (dd, 1 H, *J* = 8.0, 3.2 Hz, H-1), 4.16 – 4.08 (m, 2 H, H-6_a and H-6_b), 4.06 (dd, 1 H, *J* = 9.9, 3.6 Hz, H-2), 3.86 (t, 1 H, *J* = 9.1 Hz, H-4), 3.77 (tm, 1 H, *J* = 9.5 Hz, H-5), 3.58 (m, 3 H, $CH_{3\text{-OMe}}$), 3.49 (dd, 1 H, *J* = 10.1, 9.0 Hz, H-3), 3.34 – 3.25 (m, 8 H, $CH_{2\text{-TBA}}$), 2.03 (s, 3 H, $CH_{3\text{-NAc}}$), 1.71 – 1.59 (m, 8 H, $CH_{2\text{-TBA}}$), 1.50 – 1.38 (m, 8 H, $CH_{2\text{-TBA}}$), 1.04 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 1.00 (t, 12 H, *J* = 7.3 Hz, $CH_{3\text{-TBA}}$), 0.98 (s, 9 H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.8 ($C{=}O_{NAc}$), 93.6 (d, *J* = 4.5 Hz, C-1), 80.8 (C-3), 78.6 (C-4), 67.1 (C-5), 66.9 (C-6), 60.1 ($CH_{3\text{-OMe}}$), 58.5 ($CH_{2\text{-TBA}}$), 52.5 (d, *J* = 5.2 Hz, C-2), 27.3, 26.9 ($CH_{3\text{-tBu-Si}}$ x2), 23.8 ($CH_{2\text{-TBA}}$), 23.2 ($CH_{3\text{-NAc}}$), 22.4, 19.7 ($C_{q\text{-tBu-Si}}$ x2), 19.4 ($CH_{2\text{-TBA}}$), 13.5 ($CH_{3\text{-TBA}}$); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 0.19 (s, 1 P); IR (neat): 3271, 2963, 2862, 1652, 1473, 1084, 826, 651 cm^{-1}; HRMS Calcd for $[C_{17}H_{34}NO_9PSi + H]^+$: 456.1819, found: 456.1813.

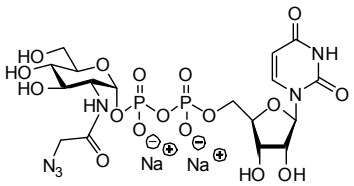


2-Acetamido-2,3-di-deoxy-4,6-*O*-di-*tert*-butylsilanediyl-*α*-D-glucopyranosylphosphate, mono-tetrabutylammonium salt (14c). The anomeric dibenzylphosphotriester **13c** (0.16 g, 0.27 mmol, 1.0 eq) was dissolved in methanol (6 mL) and purged with argon. Palladium on charcoal (Pd/C, 10 mol%) (21 mg, 0.02 mmol, 0.07 eq) was added and the reaction was purged with hydrogen gas and then stirred under a hydrogen atmosphere for three hours at ambient temperature. The mixture was filtered over a Whatmann paper and the filter was rinsed with methanol (20 mL). Concentration of the organics under reduced pressure followed by addition of 40% aq. solution of tetrabutylammonium hydroxide (0.17 mL 0.27 mmol 1.0 eq. based on dry sugar phosphate) and subsequent lyophilization, afforded the title compound as a white hygroscopic compound (0.11 g, 0.26 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.39 (dd, 1 H, *J* = 8.4, 3.0 Hz, H-1), 4.10 – 4.00 (m, 2 H, H-6_a and H-2), 3.93 (ddd, 1 H, *J* = 9.8, 9.7, 4.7 Hz, H-5), 3.85 (ddd, 1 H, *J* = 11.0, 9.3, 4.2 Hz, H-4), 3.74 (t, 1 H, *J* = 9.8 Hz, H-6_b), 3.33 – 3.27 (m, 8 H, $CH_{2\text{-TBA}}$), 2.27 (ddd, 1 H, *J* = 11.5, 4.3, 4.2 Hz, H-3_{eq}), 1.99 (s, 3 H, $CH_{3\text{-NAc}}$), 1.81 (q, 1 H, *J* = 11.6 Hz, H-3_{ax}), 1.69 – 1.61 (m, 8 H, $CH_{2\text{-TBA}}$), 1.48 – 1.41 (m, 8 H, $CH_{2\text{-TBA}}$), 1.02 (s, 9 H, $CH_{3\text{-tBu-Si}}$), 1.00 (t, 12 H, *J* = 7.4 Hz, $CH_{3\text{-TBA}}$), 0.97 (s, 9 H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.4 ($C{=}O_{NAc}$), 92.0 (d, *J* = 3.8 Hz, C-1), 72.4 (C-4), 68.6 (C-5), 67.4 (C-6), 58.71

($CH_{2\text{-}TBA}$), 49.00 (d, *J* = 4.5 Hz, C-2), 33.3 (C-3), 27.5, 27.1 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 24.0 ($CH_{2\text{-}TBA}$), 23.1 ($CH_{3\text{-}NAc}$), 22.6, 19.9 ($C_{q\text{-}tBu\text{-}Si}$ x2), 19.6 ($CH_{2\text{-}TBA}$), 13.6 ($CH_{3\text{-}TBA}$); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 0.87 (s, 1 P); IR (neat): 3271, 2964, 2862, 1660, 1473, 1103, 827, 653 cm^{-1}; HRMS Calcd for $[C_{16}H_{32}NO_8PSi+Na]^+$: 448.1527, found: 448.1526.

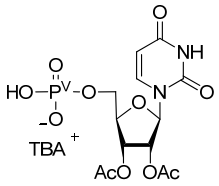


2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-*α*-D-glucopyranosylphosphate, mono-tetrabutylammonium salt (17) Following the literature procedure of Vocadlo et al.,[31] was diallyl (2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-*α*-D-glucopyranosyl) phosphate (144 mg, 0.26 mmol, 1.0 eq) transformed into the free 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-*α*-D-glucopyranosylphosphate after purification with ion-exchange chromatography (46 mg, 0.097 mmol 37%). ^{1}H-NMR (400 MHz, D_2O) δ: 5.54 (dd, 1 H, *J* = 6.9, 3.3 Hz, H-1), 5.37 (t, 1 H, *J* = 10.0 Hz, H-3), 5.16 (t, 1 H, *J* = 9.8 Hz, H-4), 4.47–4.36 (m, 3 H, H-2, H-5, H-6a), 4.20 (d, 1 H, *J* = 12.5 Hz, H-6b), 4.06 (d, 1 H, *J* = 16.3 Hz, $H_{CH2\text{-}NAcN3}$), 3.98 (d, 1 H, *J* = 16.3 Hz, $H_{CH2\text{-}NAcN3}$), 2.14 (s, 3 H, $CH_{3\text{-}OAc}$), 2.11 (s, 3 H, $CH_{3\text{-}OAc}$), 2.07 (s, 3 H, $CH_{3\text{-}OAc}$). To the lyophilized sugar phosphate was then tetrabutylammonium hydroxide (aq. 50%) (49 µL, 0.97 mmol, 1.0 eq) added, which after lyophilization produced the title compound as a white powder (63 mg, 0.89 mmol, 33% total yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, 1 H, *J* = 8.5 Hz, NH), 5.57 (bd, 1 H, *J* = 3.7 Hz, H-1), 5.33 (t, 1 H, *J* = 10.0 Hz, H-3), 5.15 (t, 1 H, *J* = 9.7 Hz, H-4), 4.38 (bt, 1 H, *J* = 9.7 Hz, H-2), 4.31 (bd, 1 H, *J* = 10.1 Hz, H-5), 4.25 (d, 1 H, *J* = 12.6 Hz, H-6a), 4.14 (d, 1 H, *J* = 12.6 Hz, H-6b), 3.98 (d, 1 H, *J* = 16.0 Hz, $CH_{2\text{-}NAcN3}$), 3.89 (d, 1 H, *J* = 16.0 Hz, $CH_{2\text{-}NAcN3}$), 3.27 (m, 8 H, $CH_{2\text{-}TBA}$), 2.04 (s, 3 H, $CH_{3\text{-}OAc}$), 1.99 (s, 3 H, $CH_{3\text{-}OAc}$), 1.97 (s, 3 H, $CH_{3\text{-}OAc}$), 1.64 (m, 8 H, $CH_{2\text{-}TBA}$), 1.44 (m, 8 H, $CH_{2\text{-}TBA}$), 1.00 (t, 12 H, *J* = 7.3 Hz, $CH_{3\text{-}TBA}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.8 ($C{=}O_{NAcN3}$), 170.6, 169.5, 168.4 ($C{=}O_{OAc}$ x3), 93.7 (d, *J* = 4.2 Hz, C-1), 71.1 (C-3), 68.3 (C-5), 68.2 (C-4), 61.6 ($CH_{2\text{-}NAcN3}$), 58.8 ($CH_{2\text{-}TBA}$), 52.0 (d, *J* = 5.8 Hz, C-2), 51.7 (C-6), 23.9 ($CH_{2\text{-}TBA}$), 20.64, 20.62, 20.5 ($CH_{3\text{-}OAc}$ x3), 19.6 ($CH_{2\text{-}TBA}$), 13.5 ($CH_{3\text{-}TBA}$); ^{31}P-NMR (162 MHz, $CDCl_3$) δ: −1.08 (s, 1 P).



Uridine 5′-(2-azidoacetamido-2-deoxy-*α*-D-glucopyranosyl diphosphate) disodium salt (18) The title compound was either synthesized as described in the general procedure for the synthesis of sugar nucleotides, except for the deprotection sequence which does not include an fluoride-treatment in this reaction sequence. Phosphoramidite **7** (31 mg, 0.058 mmol) was reacted with sugar phosphate **17** (45 mg, 0.063 mmol, 1.1 eq) producing the title sugar nucleotide as a white solid (25 mg, 0.036 mmol, 63%). The one-pot synthesis of **18**, starting from *N*-azidoacetyl glucosamine hemiacetal **21** was performed according to the following procedure: hemiacetal **21** (55 mg, 0.14 mmol, 1.25 eq) was coevaporated with anhydrous toluene (5 mL) and dissolved in anhydrous THF (2 mL) under an atmosphere of argon. The reaction mixture was cooled to −78 °C and *n*-butyllithium (1.6 M in hexanes) (88 µL, 0.14 mmol, 1.25 eq) was added. The reaction was then stirred for 15 minutes and 2-cyanoethyl-*N*,*N*-diisopropylamidochloridophosphite[28] (31 µL, 0.14 mmol, 1.25 eq) was consecutively added via syringe. The reaction mixture was left to reach room temperature (2 hours). Uridine phosphate tetrabutylammonium salt **20** (74 mg, 0.11 mmol, 1.0 eq) and dicyanoimidazole (33 mg, 0.28 mmol, 2.5 eq) was coevaporated once in anhydrous acetonitrile (4 mL) and was then dissolved in anhydrous acetonitrile (1 mL) under an atmosphere of argon. The *N*-azidoacetyl glucosamine phosphoroamidite solution was then added to the uridine phosphate at ambient temperature, by the aid of a syringe. The reaction was run for 45 minutes and the phosphate-phosphite intermediate was then oxidized with *tert*-butylhydroperoxide (5 M in decane) (112 µL, 0.56 mmol, 5 eq). The oxidation was run for 30 minutes before deprotection was initiated by the

addition of DBU (169 µL, 1.12 mmol, 10 eq). The reaction was stirred for 30 minutes and was then treated with a 1:3:1 mixture of Et_3N:MeOH:H_2O (5 mL) and left stirring over night at ambient temperature. The reaction mixture was then concentrated *in vacuo* and purified by means of size-exclusion and RP-HPLC. The purified and lyophilized product was transferred into the sodium form by dissolving the product in water and flushed through a plug of wet Amberlite Na^+ (2 mL). The water was then removed by lyophilization giving the title compound as a white solid (32 mg, 46 µmol, 42%). ^{1}H NMR (400 MHz, D_2O) δ: 7.93 (d, 1 H, *J* = 8.1 Hz, H-6), 5.98 – 5.93 (m, 2 H, H-1′ and H-5), 5.52 (dd, 1 H, *J* = 7.3, 3,3 Hz, H-1′′), 4.38 – 4.32 (m, 2 H, H-2′ and H-3′), 4.27 (m, 1 H, H-4′), 4.23 (ddd, 1 H, *J* = 11.7, 4.7, 2.6 Hz, H-5$_a$′), 4.17 (ddd, 1 H, *J* = 11.7, 5.6, 3.1 Hz, H-5$_b$′), 4.14 (d, 1 H, *J* = 16.2 Hz, $CH_{2a\text{-}N\text{-}AzAc}$), 4.08 – 4.02 (m, 2 H, $CH_{2b\text{-}N\text{-}AzAc}$ and H-2′′), 3.92 (m, 1 H, H-4′′), 3.86 (dd, 1 H, *J* = 14.9, 2.5 Hz, H-6$_a$′′), 3.84 – 3.76 (m, 2 H, H-3′′ and H-6$_a$′′), 3.55 (t, 1 H, *J* = 9.6 Hz); ^{13}C NMR (101 MHz, D_2O) δ: 174.6 ($C{=}O_{NAc}$), 166.4 (C=O C-4), 152.0 (C=O, C-2), 141.7 (C-6), 102.7 (C-5), 94.7 (d, *J* = 6.1 Hz, C-1′′), 88.5 (C-1′), 83.3 (d, *J* = 9.1 Hz, C-4′), 80.7 (C-3′′), 73.9 (C-2′), 73.1 (C-5′′), 69.7 (C-3′), 68.8 (C-4′′), 65.1 (d, *J* = 5.4 Hz, C-5), 60.3 (C-6′′), 59.6 ($CH_{3\text{-}OMe}$), 52.6 (d, *J* = 8.6 Hz, C-2′′), 22.2 ($CH_{3\text{-}NAc}$); ^{13}C NMR (101 MHz, D_2O) δ: 170.9 ($C{=}O_{N\text{-}AzAc}$), 166.6 (C-4), 152.1 (C-2), 141.6 (C-6), 102.6 (C-5), 94.4 (d, *J* = 6.3 Hz, C-1′′), 88.5 (C-1′), 83.1 (d, *J* = 9.1 Hz, C-4′), 73.7 (C-2′), 73.0 (C-4′′), 70.8 (C-3′′), 69.6 (C-3′), 69.4 (C-5′′), 65.0 (d, *J* = 5.4 Hz, C-5′), 60.3 (C-6′′), 53.7 (d, *J* = 8.6 Hz, C-2′′), 51.6 ($CH_{2\text{-}N\text{-}AzAc}$); ^{31}P NMR (162 MHz, D_2O) δ: −10.86 (d, 1 P, *J* = 21.1 Hz), -12.61 (d, 1 P, *J* = 21.1 Hz); HRMS Calcd for $[C_{17}H_{26}N_6O_{17}P_2+H]^+$: 649.0902, found 649.0903.



Uridine 2′,3′-Di-*O*-acetyl-5′-*O*-phosphate mono-tetrabutyl-ammonium salt (20) 2′,3′-Di-*O*-acetyl-uridine **5** (0.99 g, 3.0 mmol, 1.0 eq) and dicyanoimidazole (0.64 g, 5.4 mmol, 1.8 eq) were coevaporated in anhydrous acetonitrile (20 mL) and dissolved in anhydrous acetonitrile (15 mL). *N*,*N*-Diisopropylamino-di-*O*-(4-methoxybenzyl) phosphoramidite[35] (1.46 g, 3.6 mmol, 1.2 eq) was added at ambient temperature under an atmosphere of argon. The reaction was stirred for 30 minutes and *tert*-butylhydroperoxide (5 M in decane) (1.8 mL, 9 mmol, 3.0 eq) was added and the mixture was stirred for an additional 30 minutes. The reaction mixture was then concentrated in vacuo and coevaporated two times with anhydrous toluene (20 mL). The residue was then dissolved in anhydrous DCM (30 mL) cooled to 0 °C and TFA (0.9 mL) was added. The reaction was stirred for 5 minutes and anhydrous toluene (20 mL) was added and the reaction mixture was concentrated to dryness in vacuo. The residue was put on column and eluted with (20–40% MeOH in DCM). The fractions containing the product were evaporated to dryness, dissolved in water and run over a plug of wet Amberlite H^+ (6 mL). The water was removed by lyophilization and the resulting dry white powder (400 mg, 0.98 mmol) was dissolved in water (5 mL) and tetrabutylammonium hydroxide (55% in water) (470 µL, 0.98 mmol, 1.0 eq. based on dry phosphate) was added. The water was then removed by lyophilization giving the title compound as a white solid (648 mg, 0.98 mmol, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, 1 H, *J* = 8.1 Hz, H-6), 6.12 (d, 1 H, *J* = 5.3 Hz, H-1′), 5.91 (d, 1 H, *J* = 8.1 Hz, H-5), 5.45 – 5.39 (m, 2 H, H-2′ and H-3′), 4.46 (m, 1 H, H-4′), 4.16 (ddd, 1 H, *J* = 11.6, 4.5, 2.3 Hz, H-5a′), 4.10 (m, 1 H, H-5$_b$′), 3.15 (m, 8 H, $CH_{2\text{-}TBA}$), 2.14 (s, 3 H, $CH_{3\text{-}OAc}$), 2.08 (s, 3 H, $CH_{3\text{-}OAc}$), 1.60 (m, 8 H, $CH_{2\text{-}TBA}$), 1.31 (m, 8 H, $CH_{2\text{-}TBA}$), 0.90 (t, 12 H, *J* = 7.4 Hz, $CH_{3\text{-}TBA}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.8, 172.6 ($C{=}O_{OAc}$ x2), 166.1 (C-4), 151.6 (C-2), 141.7 (C-6), 102.9 (C-5), 87.0 (C-1′), 81.3 (C-4′), 73.4 (C-2′), 70.9 (C-3′), 64.5 (C-5′), 58.8 ($CH_{2\text{-}TBA}$), 23.9 ($CH_{2\text{-}TBA}$), 20.0, 19.8 ($CH_{3\text{-}OAc}$ x2), 19.6 ($CH_{2\text{-}TBA}$), 13.5 ($CH_{3\text{-}TBA}$); ^{31}P-NMR (162 MHz, $CDCl_3$) δ: 0 .3 (s, 1 P). HRMS Calcd for $[C_{13}H_{17}N_2O_{11}P+H]$+: 409.0643, found 409.0642.

References and footnotes

1. Turnbull, J. E.; Field, R. A. *Nat. Chem. Biol.* **2007,** *3* (2), 74-77.
2. Watkins, W. M. *Carbohydr. Res.* **1986,** *149* (1), 1-12.
3. After Luis F. Leloir, who discovered the first sugar nucleotide and who received the Nobel Prize in Chemistry (1970) for his investigations of carbohydrate metabolism.
4. Coutinho, P. M.; Deleury, E.; Davies, G. J.; Henrissat, B. *J. Mol. Biol.* **2003,** *328* (2), 307-317.
5. The glycosyltransferase family server at: http://www.cazy.org/GlycosylTransferases.html (accessed Aug 30, 2010)
6. Ohtsubo, K.; Marth, J. D. *Cell* **2006,** *126* (5), 855-867.
7. Hancock, S. M.; D Vaughan, M.; Withers, S. G. *Curr. Opin. Chem. Biol.* **2006,** *10* (5), 509-519.
8. Blanchard, S.; Thorson, J. S. *Curr. Opin. Chem. Biol.* **2006,** *10* (3), 263-271.
9. Prescher, J. A.; Bertozzi, C. R. *Cell* **2006,** *126* (5), 851-854.
10. Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005,** *1* (1), 13-21.
11. Khidekel, N.; Arndt, S.; Lamarre-Vincent, N.; Lippert, A.; Poulin-Kerstien, K. G.; Ramakrishnan, B.; Qasba, P. K.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2003,** *125* (52), 16162-16163.
12. Yeager, A. R.; Finney, N. S. *J. Org. Chem.* **2005,** *70* (4), 1269-1275.
13. Unless the purpose of the modified sugar-nucleotide is to inhibit or otherwise manipulate the activity of the glycosyltransferase.
14. Zhao, G.; Guan, W.; Cai, L.; Wang, P. G. *Nat. Protocols* **2010,** *5* (4), 636-646.
15. Bulter, T.; Elling, L. *Glycoconjugate J.* **1999,** *16* (2), 147-159.
16. Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1958,** *80* (14), 3756-3761.
17. Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1961,** *83* (3), 649-658.
18. Wittmann, V.; Wong, C.-H. *J. Org. Chem.* **1997,** *62* (7), 2144-2147.
19. Wagner, G. K.; Pesnot, T.; Field, R. A. *Nat. Prod. Rep.* **2009,** *26* (9), 1172-1194.
20. Timmons, S. C.; Jakeman, D. L. *Org. Lett.* **2007,** *9* (7), 1227-1230.
21. Uchiyama, T.; Hindsgaul, O. *J. Carbohydr. Chem.* **1998,** *17* (8), 1181-1190.
22. Ernst, C.; Klaffke, W. *J. Org. Chem.* **2003,** *68* (14), 5780-5783.
23. Arlt, M.; Hindsgaul, O. *J. Org. Chem.* **1995,** *60* (1), 14-15.
24. Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J. Y. *Methods Enzymol.* **1987,** *154*, 287-313.
25. DeAngelis, P. L. *Cell. Mol. Life Sci.* **1999,** *56* (7-8), 670-682.
26. Danac, R.; Ball, L.; Gurr, S. J.; Fairbanks, A. J. *Carbohydr. Res.* **2008,** *343* (6), 1012-1022.
27. Filippov, D.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; Tesser, G. I.; van Boom, J. H. *Tetrahedron Lett.* **1998,** *39* (21), 3597-3600.
28. Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984,** *12* (11), 4539-4557.
29. Vargeese, C.; Carter, J.; Yegge, J.; Krivjansky, S.; Settle, A.; Kropp, E.; Peterson, K.; Pieken, W. *Nucleic Acids Res.* **1998,** *26* (4), 1046-1050.
30. Anhydrous conditions were used to prevent possible hydrolysis of the presumably labile phosphite-phosphate anhydride **15a**.
31. Vocadlo, D. J.; Hang, H. C.; Kim, E. J.; Hanover, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U. S. A.* **2003,** *100* (16), 9116-9121.
32. Marlow, A. L.; Kiessling, L. L. *Org. Lett.* **2001,** *3* (16), 2517-2519.
33. Or 23% overall yield starting from 3,4,6-tetra-*O*-acetyl-2-azidoacetamido-2-deoxy-α-D-glucopyranose **21**.
34. Serwa, R.; Wilkening, I.; Del Signore, G.; Muhlberg, M.; Claussnitzer, I.; Weise, C.; Gerrits, M.; Hackenberger, C. P. R. *Angew. Chem., Int. Ed.* **2009,** *48* (44), 8234-8239.

35. van der Heden van Noort, G. J.; Verhagen, C. P.; van der Horst, M. G.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V. *Org. Lett.* **2008,** *10* (20), 4461-4464.
36. The occurrence of an intramolecular reaction between an azide and a phosphoramidite have been indicated in a publication by Jawalkar *et al*. *J. Org. Chem.* **2008,** *73* (1), 287-290. Isolation of a nucleobase containing the mentioned groups rendered a relatively low yield as compared to isolation of the nucleobase equipped with an alkyne instead of an azide.
37. Jawalekar, A. M.; Meeuwenoord, N.; Cremers, J. G. O.; Overkleeft, H. S.; van der Marel, G. A.; Rutjes, F.; van Delft, F. L. *J. Org. Chem.* **2008,** *73* (1), 287-290.
38. Condensation was performed according to the optimized conditions for the pyrophosphate formation from phosphoramidites.
39. Kenner, G. W.; Todd, A. R.; Webb, R. F.; Weymouth, F. J. *J. Chem. Soc.* **1954**, 2288-2293.

Chapter 7

Summary and Future Prospects

Carbohydrate processing enzymes are essential for a plethora of biological functions and processes. This thesis has focussed on the synthesis of molecules that have the potential of being used for either the monitoring or modification of some of these processes. Detailed information about the various functions and purposes of the enzymes and intermediates in metabolic pathways may result in the development of novel pharmaceutical drugs or aid the monitoring of pathological processes in patients.

Chapter 1 of this thesis described some basic functions of carbohydrate modifying enzymes and the different techniques by which these enzymes can be manipulated or monitored. There are in principal two different types of carbohydrate modifying enzymes, the ones that build carbohydrate structures or conjugates (i.e. glycosyltransferases) and the ones that disassemble these structures (i.e. glycosidases). There are a number of ways that these enzymes can be manipulated and/or monitored. Chemical probes can be used to both inhibit and label carbohydrate processing enzymes to provide information about substrate specificity and activity of the enzymes. Modification of glycosyltransferases and/or their

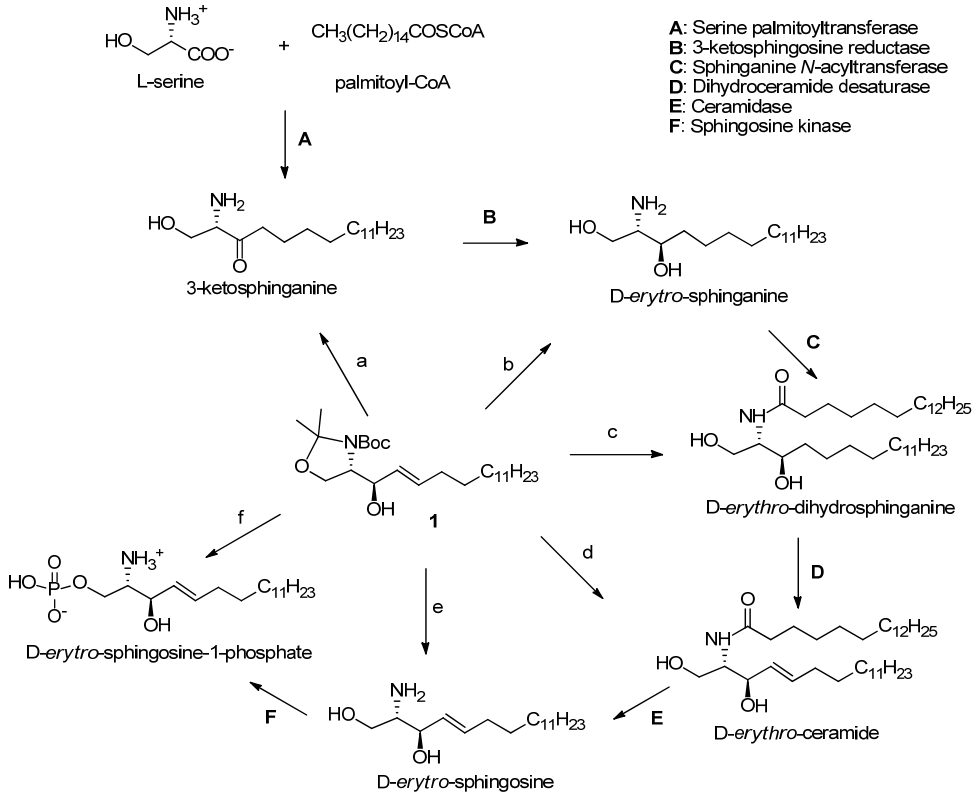
substrates can either be utilized for the assembly of non-natural glycoconjugates or attachment of labels/effectors for the investigation of the different sites and functions of specific glycosylation events.

A novel and robust synthetic strategy for the assembly of globotriaosylsphingosine was presented in **Chapter 2**. The 4,6-di-*tert*-butylsilylene group was utilized as a protecting group for the non-reducing end galactose that enabled both complete stereoselectivity in the formation of the D-Gal-*α*-D-Gal glycosidic linkage and a mild deprotection of the final product. Exploration of the coupling of the protected globotriaosyl donors with azido-sphingosine resulted in the formation of the protected globotriaosylsphingosine in 57% yield over four steps from a galactose imidate donor. Subsequent deprotection and purification by RP–HPLC–MS produced the glycosphingolipid in an overall good yield. **Chapter 3** described the development of $^{13}C_5$-labeled lysoCTH, which can be used as an internal standard for the monitoring of lysoCTH levels in Fabry patients. Synthesis of the $^{13}C_5$-labeled D-*erythro*-sphingosine was accomplished *via* an efficient formation of a ^{13}C-labeled C_2-elongation reagent. After an initial substitution of nonylbromide with $^{13}C{\equiv}N$, the C_2-elongation reagent was utilized in two consecutive Horner-Wadsworth-Emmons reactions for the synthesis of a $^{13}C_5$-labeled fatty olefin. The hydrophilic part of the sphingosine was derived from the reaction of Garner's aldehyde with vinyl lithium creating the desired D-*erythro*-configuration. With the two olefins in hand a cross-metathesis reaction utilizing Grubbs^{II}-ruthenium catalyst was performed. This reaction did not only produce the desired D-*erythro*-sphingosine, but also two additional nor- and di-nor-homologues. This unexpected isomerization and elimination process lowered the yield and efficiency of the synthesis and further investigation should be made to optimize this reaction step. The use of additives, change in catalyst or the use of different solvents has been shown to minimize these side reactions in ring closing metathesis reactions.[1-3] The assembled $^{13}C_5$-labeled D-*erythro*-sphingosine was then glycosylated using a globotriaosyl *N*-phenyl trifluoroacetimidate already utilized in **Chapter 2**. Following a mild deprotection sequence, the pure $^{13}C_5$-labeled lysoCTH and the individual homologues were isolated using RP-HPLC.

The availability of different labeled (glyco-)sphingolipids can both improve and simplify the diagnosis and monitoring of patients with a variety of sphingolipidoses. Furthermore, with a set of labeled (glyco-)sphingolipids in store, research can be performed to further investigate these disorders and their pathology for the development of new pharmaceutical agents. In line of the work presented in **Chapter 3**, numerous $^{13}C_5$-labeled sphingolipids can be produced that are involved in the biosynthetic pathway of sphingosine[4] (Scheme 1). For instance, catalytic hydrogenation and deprotection of sphingosine **1** gives D-*erythro*-sphinganine. Oxidation of the C3-OH of sphinganine followed by deprotection gives 3-ketosphinganine, which corresponds to the product formed by the condensation of L-serine and palmitoyl-CoA in the biosynthetic pathway. Deprotection of the partially protected D-*erythro*-sphingosine **1** followed by acylation of the resulting amine with the *N*-hydroxysuccinimide ester of steric acid, gives access to both D-*erythro*-ceramide

and D-*erythro*-dihydroceramide after hydrogenation of the double bond. Protection of C3-OH in sphingosine as a PMB-ether followed by acetonide deprotection and phosphorylation[5] of the resulting primary alcohol would produce the important signalling lipid D-*erythro*-sphingosine-1-phosphate after TFA-deprotection. Furthermore, glycosylation of the labeled sphingolipids can produce a wide variety of labeled glycosphingolipids, corresponding to the natural substrates that are accumulated in patients suffering from different sphingolipidoses. Important examples include glucosylceramide (Gaucher) or galactosylceramide and galactosylsphingosine also known as psychosine (Krabbe).

Scheme 1. *De novo* biosynthesis of sphingolipids and synthesis of their ($^{13}C_5$-labeled) counterparts.



Reagents and conditions: [a] i) Pd/C, H_2, EtOAc, r.t.; ii) DMSO, oxalylchloride, Et_3N, −78 °C; iii) TFA (neat), 0 °C; [b] i) Pd/C, H_2, EtOAc, r.t., ii) TFA (neat), 0 °C; [c] i) Pd/C, H_2, EtOAc, r.t.; ii) TFA (neat), 0 °C; iii) Stearic acid *N*-hydroxysuccinimide ester, THF, r.t.; [d] i) TFA (neat), 0 °C; ii)[6] Stearic acid *N*-hydroxysuccinimide ester, THF, r.t.; [e] TFA (neat), 0 °C; [f] i) PMBCl, NaH, THF, r.t.; ii) $AcOH_{aq}$ 80%, 80 °C; iii) $(PMB)_2PN(^iPr)_2$, DCI, MeCN, r.t., then ^{t}BuOOH; iv) TFA (neat), 0 °C.

In **Chapter 4** three different dimeric hyaluronic acid probes bearing an anomeric *β*-4-methylumbelliferone were synthesized. A 4,6-*O*-di-*tert*-butylsilylene protecting group was introduced to GlcNAc-*β*-4MU producing a building block with good solubility, which proved to be an excellent acceptor in condensation reactions utilizing

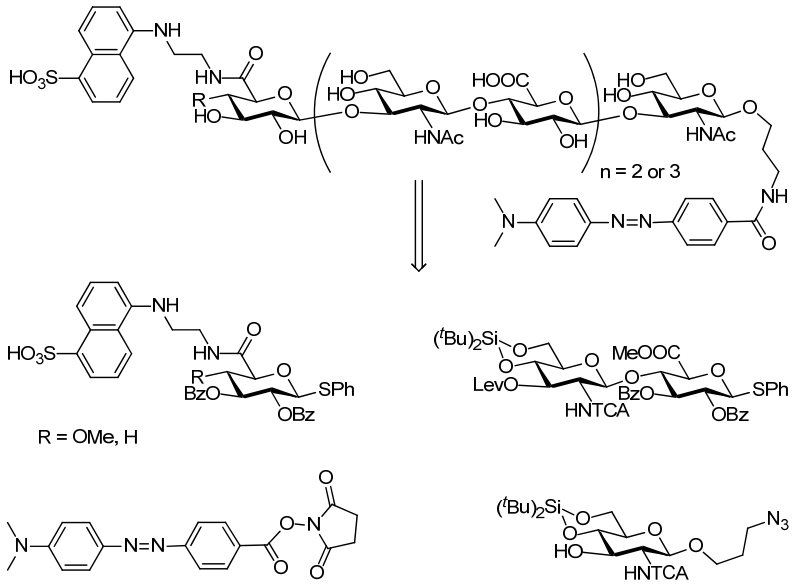
either imidate or dehydrative coupling procedures. This route enabled the use of GlcNAc as building block avoiding the need for a temporary amine protective group. The GlcNAc-*β*-4MU acceptor was condensed with three different functionalized glucuronic acid donors via a dehydrative condensation strategy. The hyaluronic acid dimers were deprotected, purified and evaluated in a hyaluronidase assay. The investigated probes proved inactive towards the hyaluronidase enzyme, but were successively processed by two *exo*-glycosidase enzymes (i.e. *exo*-*β*-glucuronidase and *exo*-*β*-*N*-acetylglucosaminidase) producing a fluorometric read-out.

Chapter 5 describes the synthesis of two hyaluronic acid tetramers bearing the anomeric *β*-4MU fluorophore. These molecules were synthesized in an attempt to produce probes with sufficient recognition surface to function as substrates for the selected hyaluronidases. Key building block was an *N*-TCA protected D-glucosamine-*β*-D-glucuronic acid donor. This dimer was synthesized utilizing an *N*-TCA protected D-glucosamine imidate donor obtained in four steps from D-glucosamine hydrochloride, in a condensation reaction with a known phenyl 1-thio-glucuronic acid acceptor. The *N*-TCA protected hyaluronic acid dimer was then stepwise elongated by condensation reactions with the respective glucuronic acid and GlcNAc-4MU monomers described in **Chapter 4** for the assembly of the two different C4‴-modified hyaluronic acid derivatives. The hyaluronic acid tetramers were deprotected by an initial transformation of the *N*-TCA moiety into the corresponding *N*-acetyl derivative by zinc/acetic acid mediated dehalogenation. The deprotection was then finalized following the sequence already developed for the dimeric hyaluronidase probes in **Chapter 4**. Purification by RP–HPLC afforded two tetrameric hyaluronic acid probes, which also proved inactive towards the bovine PH20 and human Hyal2 hyaluronidases.

The degradation of hyaluronan by hyaluronidases proceeds through a complex mechanism, which entails both hydrolase and transglycosidase activities (Chapter 1). The exact mechanisms behind these transformations are not known, which makes the development of hyaluronidase probes a challenging task. A crystal structure of the hyaluronidase from bee venom has been resolved with a hyaluronic acid tetramer bound at the −4 to −1 subsite of the active site.[7] The substrate is bound along a groove in the enzyme exposing it to the catalytic residues. Experimental data show that larger hyaluronic acid oligomers have a higher affinity for the hyaluronidase suggesting that probes need recognition at both sides of the scissile glycosidic linkage for optimal activity. In light of this, a functional hyaluronidase probe utilizing an anomeric fluorophore might be suboptimal. An alternative probe is proposed that is unsensitive for the cleavage site of the glycosidic linkage by the use of FRET (Scheme 2).

Synthesis of a hexameric or octameric hyaluronan substrate equipped with the commonly used FRET donor/quencher pair (EDANS/DABCYL) at the reducing and non-reducing end respectively, may provide an effective probe for the measuring of hyaluronidase activity in a continuous fashion.

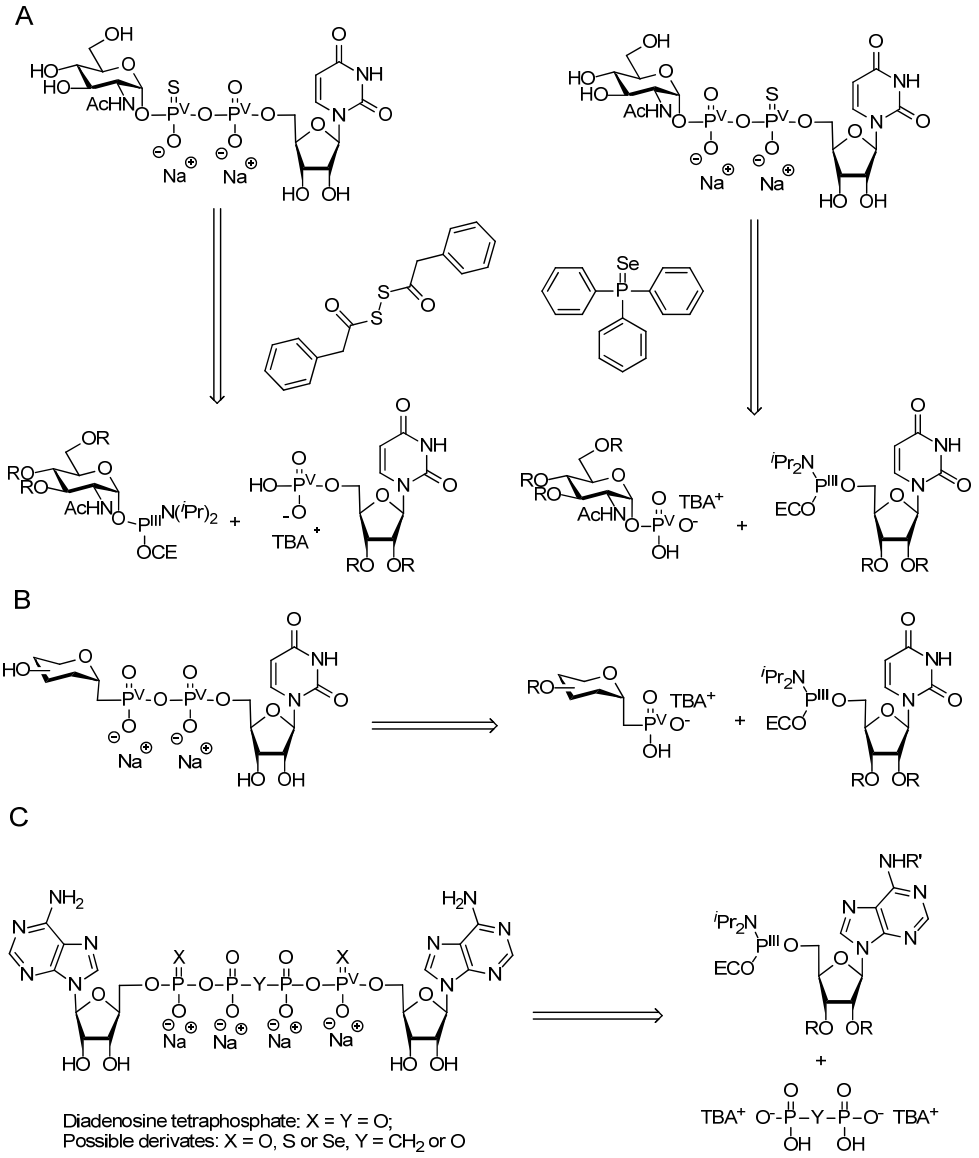
Scheme 2. Retrosynthesis of potential probes for the measuring of hyaluronidase activity using FRET.



Chapter 6 describes the development of a novel methodology for the preparation of pyrophosphate moieties present in sugar nucleotides.[8] The methodology utilized the highly potent phosphorylating properties of phosphoramidites in a reaction with anomerically predefined sugar phosphates. Activation of the nucleoside phosphoramidite and condensation with the tetrabutylammonium salt of a sugar phosphate gave a phosphate-phosphite intermediate that was oxidized and deprotected in a one-pot fashion.[9] This methodology was utilized for the synthesis of a number of modified glucosamine sugar nucleotides. For the synthesis of an *N*-azidoacetyl glucosamine sugar nucleotide, a reversed approach was developed in which the desired target compound was acquired in a one-pot synthesis starting from the corresponding sugar hemiacetal. This was accomplished by introduction of a phosphoramidite at the anomeric position of the sugar, followed by condensation with the tetrabutylammonium salt of a nucleoside monophosphate.

It is proposed that the developed methodologies are also suitable for the synthesis of phosphate analogues, such as phosphorothioates that can be used in a wide range of biochemical applications.[10] Replacement of the non-bridging oxygen of the phosphate group of nucleotides by sulphur introduces a chiral centre at the phosphor. Such chiral phosphorothioates can be utilized for the investigation of stereochemical effects in enzymatic reactions. The two methodologies for the synthesis of sugar nucleotides presented in **Chapter 6** are characterized by the intermediate formation of a P^{III} species at either the anomeric C1″-OH or the nucleoside C5′-OH position. This makes the synthesis of phosphorothioates at eiter position possible by application of sulphurizating agents such as phenacetyl disulfide[11] (Scheme 3, **A**).[12] Additionally, application of triphenylphosphine selenide may provide the corresponding phosphoroselenoate derivatives.[13]

Scheme 3. Retrosynthesis of various (sugar)nucleotide analogues.



Phosphono-analogues of sugar nucleotides represent another modification of the pyrophosphate moiety that give rise to a class of molecules that have been applied for the development of antiretroviral drugs, potential inhibitors of bacterial transglycosidase activity and for the study of enzymes involved in phosphate bond cleavage.[14,15] It is proposed that these compounds are accessible by application of the phosphoramidite methodology in combination with carbohydrate C-phosphonates as substrates (Scheme 3, **B**).

Diadenosine tetraphosphate is one of the dinucleoside polyphosphates that have been discovered in various organisms. The cellular levels of these polyphosphates are often elevated when the organism is exposed to stress.[16] The tetraphosphates and derivatives thereof are accessible by phosphitylation of a pyrophosphate substrate (or analogue thereof) with an excess of activated adenosine phosphoramidites, followed by oxidation using any of the oxidizing agents described above (Scheme 3, **C**).

References and footnotes

1. Hong, S. H.; Sanders, D. P.; Lee, C. W.; Grubbs, R. H. *J. Am. Chem. Soc.* **2005,** *127* (49), 17160-17161.
2. Bourgeois, D.; Pancrazi, A.; Nolan, S. P.; Prunet, J. *J. Organomet. Chem.* **2002,** *643*, 247-252.
3. Adjiman, C. S.; Clarke, A. J.; Cooper, G.; Taylor, P. C. *Chem. Commun.* **2008,** (24), 2806-2808.
4. van Echten, G.; Sandhoff, K. *J. Biol. Chem.* **1993,** *268* (8), 5341-5344.
5. van der Heden van Noort, G. J.; Verhagen, C. P.; van der Horst, M. G.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V. *Org. Lett.* **2008,** *10* (20), 4461-4464.
6. Julina, R.; Herzig, T.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1986,** *69* (2), 368-373.
7. Markovic-Housley, Z.; Miglierini, G.; Soldatova, L.; Rizkallah, P. J.; Müller, U.; Schirmer, T. *Structure* **2000,** *8* (10), 1025-1035.
8. Gold, H.; van Delft, P.; Meeuwenoord, N.; Codee, J. D. C.; Filippov, D. V.; Eggink, G.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2008,** *73* (23), 9458-9460.
9. Gold, H.; Descroix, K.; Codee, J. D. C.; van der Marel, G. A., *Synthesis of Sugar nucleotides: A Phosphoramidite Approach*. CRC Press Inc: 2011; Vol. Volume 1, p 500.
10. Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983,** *22* (6), 423-439.
11. Kamer, P. C. J.; Roelen, H. C. P. F.; van den Elst, H.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1989,** *30* (48), 6757-6760.
12. In an NMR-experiment the condensation between a nucleoside phosphoramidite and a GlcNAc-α-phosphate was accompanied by sulphurization with the phenacetyl disulfide reagent. This produced an ^{31}P-NMR indicative of the formation of a pyrophosphothioate.
13. Bollmark, M.; Stawinski, J. *Chem. Commun.* **2001,** (8), 771-772.
14. Beaton, S. A.; Huestis, M. P.; Sadeghi-Khomami, A.; Thomas, N. R.; Jakeman, D. L. *Chem. Commun.* **2009,** (2), 238-240.
15. Brooks, G.; Edwards, P. D.; Hatto, J. D. I.; Smale, T. C.; Southgate, R. *Tetrahedron* **1995,** *51* (29), 7999-8014.
16. Guranowski, A. *Acta Biochim. Pol.* **2003,** *50* (4), 947-972.

Nederlandse Samenvatting

Moleculair gereedschap voor het onderzoek naar het metabolisme van polysacchariden en glycolipiden

Koolhydraten zijn niet alleen belangrijk als energiebron, maar vervullen tevens een cruciale rol in tal van vitale biologische processen. Koolhydraten, aanwezig aan de buitenkant van de celwand, zijn belangrijk voor de onderlinge herkenning van cellen en hun communicatie met de buitenwereld. Koolhydraatstructuren, die kenmerkend zijn voor bepaalde virussen of bacteriën, worden bijvoorbeeld door het immuunsysteem herkend, waarna een proces in gang wordt gezet om de betrokken ziekteverwekkers te elimineren. Een ander voorbeeld betreft posttranslationele modificatie van eiwitten, een proces waarbij koolhydraatstructuren op eiwitten worden aangebracht en waarmee de activiteit van deze eiwitten wordt gereguleerd. Niet alleen de fysisch-chemische eigenschappen van deze eiwitten wordt hierdoor beïnvloed maar ook hun stabiliteit en drie-dimensionale structuur.

In het menselijk lichaam komen veel verschillende enzymen voor die de opbouw en afbraak van de koolhydraatstructuren regelen. Deze enzymen kunnen worden onderverdeeld in glycosyltransferasen, die koolhydraatstructuren opbouwen en glycosidasen, die deze afbreken. Daar deze enzymen betrokken zijn bij vele biologische processen, is hun werking van vitaal belang. Wanneer een enzym, dat betrokken is bij het suikermetabolisme ontbreekt of defect is, kan dit leiden tot ernstige medische aandoeningen. Het doel van het in dit proefschrift beschreven onderzoek is het ontwikkelen van nieuwe synthetische methoden en moleculair gereedschap om de activiteit van bepaalde enzymen en de vorming van hun producten te kunnen bepalen.

Hoofdstuk 1 geeft een overzicht van geselecteerde enzymen en hun functies in het koolhydraat-metabolisme. Verschillende technieken en methodologiën die ontwikkeld zijn om de activiteit van deze enzymen vast te stellen of te beïnvloeden worden besproken. Aandacht wordt besteed aan speciaal ontworpen moleculen, die kunnen worden gebruikt als moleculair gereedschap om bijvoorbeeld eiwitten en/of hun substraten aan te tonen. Op deze wijze wordt informatie verkregen over hun functie en activiteit.

Hoofdstuk 2 behandelt de ontwikkeling van een nieuwe syntheseroute naar globotriaosylsphingosine (lysoCTH), een suiker conjugaat dat waarschijnlijk de symptomen veroorzaakt die optreden bij patiënten die lijden aan de ziekte van Fabry. De primaire oorzaak van deze ziekte is het defecte enzym “α-galactosidase A”, dat de afbraak van het globotriaosylceramide bewerkstelligt. De verlaagde enzymactiviteit leidt ertoe dat dit substraat en een metaboliet genaamd globotriaosylsphingosine (lysoCTH) zich ophopen in de weefsels van patiënten, wat resulteert in de symptomen die gekoppeld zijn aan de ziekte. De ontwikkeling van de synthese van lysoCTH kon als uitgangspunt worden genomen voor een synthese van

een ^{13}C-gemerkt analoog, dat in **hoofdstuk 3** wordt beschreven. Een ^{13}C-gemerkte versie van lysoCTH kan worden gebruikt als een interne standaard voor het bepalen van de concentratie van het endogene lysoCTH in het plasma van Fabry patiënten. Het ^{13}C-gemerkt lysoCTH is een optimale interne standaard omdat deze verbinding met uitzondering van het molecuulgewicht dezelfde fysisch-chemische eigenschappen heeft als het endogene molecuul. Het ^{13}C-gemerkte lysoCTH kon worden gesynthetiseerd met behulp van goedkope ^{13}C-gemerkte uitgangsstoffen en een syntheseroute die op zijn robuustheid en rendement werd geselecteerd door middel van eenzelfde synthese met ongemerkte uitgangsstoffen. Met behulp van het gesynthetiseerde ^{13}C-gemerkt lysoCTH kan het ziekteverloop van patiënten makkelijker onderzocht worden en de medicatie per patiënt beter afgestemd worden. Dit onderzoek is in volle gang en er is met behulp van het ^{13}C-gemerkte lysoCTH een methode ontwikkeld om de hoeveelheid lysoCTH in plasma van patiënten te bepalen, met een tot op heden groots mogelijke nauwkeurigheid.

In de **hoofdstukken 4** en **5** wordt de synthese beschreven van gemodificeerde hyaluronan fragmenten die mogelijkerwijs als moleculair gereedschap kunnen dienen om enzymen te identificeren die behoren tot de groep hyaluronidasen. Deze enzymen zijn verantwoordelijk voor de afbraak van hyaluronan. Hyaluronan is een macromoleculair polysaccharide met een verscheidenheid aan functies, die gekoppeld lijken te zijn aan de grootte van het polymeer. Door het ontwikkelen van moleculen die gebruikt kunnen worden om de activiteit van hyaluronidasen te meten, kan hun betrokkenheid bij een aantal medische aandoeningen worden geconstateerd. Zowel dimeren (**hoofdstuk 4**) als tetrameren (**hoofdstuk 5**) van hyaluronan, uitgerust met een reporter-molecuul (fluorofoor), werden voor dit doel gesynthetiseerd. Het idee van deze gemodificeerde hyaluronan fragmenten is dat de enzymen het reporter-molecuul splitsen, waardoor fluorescentie ontstaat, wat door middel van spectrofotometrie gemeten kan worden. Er werd een nieuwe syntheseroute ontwikkeld die vijf verschillende hyaluronan fragmenten uitgerust met een reporter-molecuul opleverde. Twee verschillende enzymen werden getest, maar dit leverde geen positief resultaat op.

Hoofdstuk 6 beschrijft een nieuwe toepassing van een veel gebruikte fosforylerings procedure, de zogenoemde fosforamidiet methode. Het is algemeen bekend dat de fosforamidiet methode het meest efficiënt is voor de introductie van fosfodiësters zoals die DNA en RNA voorkomen. Het fosfitylerings agens dat voor de geautomatiseerde synthese van nucleïnezuren wordt toegepast werd onderzocht op toepasbaarheid voor de vorming van pyrofosfaten. Deze functionaliteit komt onder andere voor in suikernucleotiden, de natuurlijke substraten van glycosyltransferases. Vanwege hun belang in de enzymatisch bereiding van suikerstructuren staat de synthese van suikernucleotide sterk in de belangstelling. De voornaamste uitdaging in de synthese van deze moleculen is de invoering van de pyrofosfaat binding. Met behulp van de fosforamidiet methode werd een efficiënte één-pots drie-staps procedure ontwikkeld voor de introductie van pyrofosfaten. Deze procedure werd toegepast in de synthese van een aantal suikernucleotiden.

Svensk Sammanfattning

Kemiska redskap för undersökning av polysackarid- och glykolipidmetabolism

Socker är inte bara en viktig energikälla utan har många andra livsviktiga funktioner i alla biologiska system. Sockerenheter utgör bl.a. ett viktigt språk som celler kan kommunicera med omvärlden på. Ett viktigt exempel är hur specifika sockerenheter är sammankopplade med vissa virala och bakteriella infektioner, vilket medför att immunförsvaret kan känna igen och eliminera de inkräktande patogenerna. Sockerenheter påverkar dessutom de fysiokemiska egenskaperna hos de makromolekyler som de är bundna till. Exempel på detta är påverkan av proteiners löslighet, men sockerenheter påverkar också proteiners möjlighet att få en korrekt tredimensionell form samt deras stabilitet och aktivitet.

I våra kroppar finns många olika enzymer som reglerar biosyntes och nedbrytning av sockerenheter. Dessa enzym är ett krav för många biologiska funktioner och är därmed livsviktiga för varje levande organism, oavsett storlek. Enzymen som utför dessa funktioner kan delas in i glykosyltransferaser som bygger upp sockerstrukturer och glykosidaser som bryter ner dem. I de fall där ett protein som är involverat i sockermetabolism antingen saknas eller är defekt kan det leda till allvarliga sjukdomstillstånd. Denna avhandling syftar till att utveckla nya syntetiska metoder och verktyg för att kunna följa både aktivitet och funktion av specifika enzym eller deras metaboliter.

I **kapitel 1** ges en genomgång av några grundläggande funktioner hos ett par enzymgrupper som påverkar glykometabolism och de olika tekniker som använts för att följa eller påverka enzymernas funktion. Kemiskt framställda verktyg kan användas för att till exempel inhibera eller märka proteiner och/eller deras substrat för att få information om deras funktion och aktivitet.

I **kapitel 2** utvecklas en ny syntes av en biologiskt relevant molekyl som misstänks ligga bakom de symtom som uppkommer hos patienter som lider av Fabrys sjukdom. Den primära orsaken till sjukdomen är det defekta enzymet ”α-galaktosidas A”, som har till uppgift att bryta ner ett sockerkonjugat kallat globotriaosylceramid. Den nedsatta enzymaktiviteten leder till att substratet och en metabolit kallad globotriaosyl-sfingosin (lysoCTH) ackumuleras i patienternas vävnader, vilket resulterar i de symtom som är sammankopplade med sjukdomen. Utvecklingen av den nya selektiva syntesen av lysoCTH följs upp med en syntes av en ^{13}C-markerad analog i **kapitel 3**. En ^{13}C-markerad version av lysoCTH kan användas som intern standard för att bestämma koncentrationen av endogent lysoCTH i plasma från patienter. Fördelen med ^{13}C-markerad lysoCTH är att analogen har exakt samma fysiokemiska egenskaper som den endogena molekylen, med undantag av en avvikande molvikt. Detta ger analogen optimala egenskaper för att användas som just intern standard.

Den ^{13}C-markerade analogen syntetiseras med hjälp av billiga ^{13}C-markerade startmaterial och en syntesväg som utarbetats med hjälp av omarkerat material för att få till en robust syntes med högt utbyte. Med hjälp av en ^{13}C-markerad intern standard kan läkare lättare undersöka patienternas sjukdomsförlopp och underlätta en väl kalibrerad medicinering. Dessa försök pågår redan och en metod har utvecklats med hjälp av den ^{13}C-markerade interna standarden som grund, vilket har gett upphov till den mest känsliga mätningen av lysoCTH i plasma som hittills rapporterats.

I **kapitel 4** och **5** görs ett försök att syntetisera ett verktyg för att kartlägga enzymer tillhörande gruppen hyaluroinidaser. Dessa enzym har till uppgift att bryta ned hyaluronan. Hyaluronan är en makromolekylär polysackarid som har en mängd olika funktioner, vilka är beroende av polymerens storlek. Genom att tillverka ett verktyg som kan användas för att mäta aktiviteten av hyaluronidaser kan deras involvering i en mängd olika sjukdomstillstånd utrönas. I de två kapitlen tillverkas både dimerer och tetramerer av hyaluronan som är utrustade med en reportermolekyl (fluorofor). Tanken med dessa verktyg är att enzymen skall klyva reportermolekylen, vilket leder till fluorescens som kan mätas på spektofotometrisk väg. En ny syntesväg utvecklas och fem olika verktyg framställs, men ingen av dessa verktyg accepteras av de två olika testade enzymen.

Kapitel 6 beskriver en helt ny applikation för ett redan känt fosfityleringsreagens kallat fosforamidit. Detta reagens har traditionellt används för syntes av DNA och RNA med stor framgång. Dessa molekyler består av sockerenheter som är åtskilda av fosfatgrupper, vilka bildas med hjälp av just fosforamidit. Trots att en grupp av molekyler, såkallade sockernukleotider, är av stor vikt för uppbyggnaden av sockerstrukturer i alla biologiska system, har den komplicerade syntesen av dessa molekyler inneburit ett hinder för forskningen inom detta område. Till skillnad från DNA och RNA innehåller sockernukleotider socker som är åtskilda av två fosfater. Den hittills mest effektiva syntesmetoden för dessa difosfater utvecklades på 1960-talet av Khorana/Moffatt, men sedan dess har nya effektiva metoder saknats. Utgående från vetskapen om den reaktivtet som en fosforamidit besitter användes det i ett försök att tillverka difosfater, vilket visade sig vara ett mycket effektivt tillverkningssätt. Sockernukleotider kan tillverkas på mycket kort tid med den utvecklade metoden i mycket höga utbyten.

I **kapitel 7** ges förslag på hur de i denna avhandling utvecklade metoder och verktyg kan förfinas och utvecklas för att ge ännu bättre verktyg. Dessutom introduceras idéer till nya intressanta projekt inom de områden som täcks av avhandlingen.

List of Publications

Sensitive quantification of plasma globotriaosylsphingosine by LC-ESI-MS/MS with a stable isotope-labeled standard.

Gold H., Mirzaian M., Dekker N., Ferraz M.J., Lugtenburg J., Codée J.D.C., van der Marel G.A., Overkleeft H.S., Hollak C.E.M., Linthorst G.E., Aerts J.M.F.G., Groener J.E.M., Poorthuis B.J.H.M.

Manuscript in preparation.

A practical synthesis of capped 4-methylumbelliferyl hyaluronan disaccharides and tetrasaccharides as potential hyaluronidase substrates.

Gold H., Munneke S., Dinkelaar J., Overkleeft H.S., Aaerts J.M.F.G., Codée J.D.C., van der Marel G.A.

J. Carb. Res. - In Press.

Biomarkers in the diagnosis of lysosomal storage disorders: proteins, lipids, and inhibodies.

Aerts J.M.F.G., Kallemijn W.W., Wegdam W., Ferraz M.J., van Breemen M.J., Dekker N., Kramer G., Poorthuis B.J., Groener J.E.M., Cox-Brinkman J., Rombach S.M., Hollak C.E.M., Linthorst G.E., Witte M.D., Gold H., van der Marel G.A., Overkleeft H.S., Boot R.G.

J. Inherit. Metab. Dis. – In Press.

A Concise Synthesis of Globotriaosylsphingosine.

Gold H., Boot R.G., Aerts J.M.F.G., Overkleeft H.S., Codée J.D.C., van der Marel G.A.

Eur. J. Org. Chem. **2011***, 9*, 1652-1663.

Synthesis of Sugar Nucleotides: A Phosphoramidite Approach.

Gold H., Descroix K., Codée J.D.C., van der Marel G.A.

Carbohydrate Chemistry: Proven Synthetic Methods, Volume 1, Ed. Kovac P., CRC Press Inc. **2011**.

Synthesis of Hyaluronic Acid Oligomers using Chemoselective and One-Pot Strategies.

Dinkelaar J., Gold H., Overkleeft H.S., Codée J.D.C., van der Marel G.A.

J. Org. Chem. **2009***, 74,* 4208-4216.

Synthesis of Sugar Nucleotides by Application of Phosphoramidites.

Gold H., van Delft P., Meeuwenoord N., Codée J.D.C., Filippov D.V., Eggink G., Overkleeft H.S., van der Marel G.A.

J. Org. Chem. **2008***, 73,* 9458-9460*.*

Fast and Selective Synthesis of Novel Cyclic Sulfamide HIV-1 Protease Inhibitors under Controlled Microwave Heating.

Gold H., Ax A., Vrang L., Samuelsson B., Karlén A., Hallberg A., Larhed M.

Tetrahedron, **2006**, *62*, 4671-4675*.*

Microwave Irradiation as a High-Speed Tool for Activation of Sluggish Aryl Chlorides in Grignard Reactions.

Gold H., Nilsson P., Larhed M.

Synlett, **2005***, 10*, 1596-1600*.*

Microwave-Assisted Enantioselective Heck Reactions: Expediting High Reaction Speed and Preparative Convenience.

Nilsson P., Gold H., Larhed M., Hallberg A.

Synthesis, **2002***, 11*, 1611-1614*.*

Curriculum Vitae

Karl Henrik Gold was born and grew up in the city of Örebro, Sweden on 27 January 1976. After his secondary education at Rudbecksskolan, he left his home region in 1995 to study Pharmaceutical Sciences at Uppsala University.

In January 1996, Henrik took a leave of study to move to the North of Sweden for his military service in the Swedish Army, Armored Forces, awarding him with the rank of Sergeant in the spring of 1997.

Following his military service, Henrik worked as a consultant for Apoteket AB, where he compiled a survey of pharmacy customers' drug compliance. After successfully finishing the survey, he continued his pharmaceutical studies in August 1997, alongside a part-time position at the local pharmacy.

From June through December 2000, Henrik worked fulltime as a research assistant at Amersham Pharmacia Biotech on the development of novel, high performance, polystyrene-based separation media for the ÄKTA® system.

Under supervision of Prof. Dr. Anders Hallberg, Henrik completed his Master thesis entititled "Microwave-assisted Enantioselective Heck reactions" at the Medicinal Chemistry Group, Uppsala University. He obtained his MSc in Pharmacy in 2001.

From January through August 2002, Henrik worked as a pharmacist at the Örebro University Hospital, at which time he also received his license to practice pharmacy. Subsequently, he started his graduate studies with the group of Prof. Dr. Anders Hallberg, which focused on the development of homogenous metal-catalyzed reactions under microwave irradiation to be utilized in the development of novel HIV-1 protease inhibitors. During this work, Henrik developed the most active cyclic sulfonamide based HIV-1 protease inhibitor recorded to date. He finalized his graduate studies in the summer of 2005 with a Licentiate thesis entitled "Microwave promoted organometallic reactions: Preparation of Novel Cyclic HIV-1 protease inhibitors".

After acquiring his Ph. Licentiate degree in 2006, Henrik moved to the Netherlands, where he worked for a short while at the Academic Training Association, before becoming affiliated as a graduate student at Leiden University. The work presented in this thesis was conducted with funding from the B-BASIC program, at the Bio-Organic Synthesis Group of the Leiden Institute of Chemistry, under supervision of Prof. Dr. G.A. van der Marel, Prof. Dr. H.S. Overkleeft and Dr. J.D.C. Codée. Parts of this thesis have been presented by posters at the annual NWO 'design and synthesis' symposiums in Lunteren and by oral presentations at the NWO 'design and synthesis' symposium in October 2008 and at the HRSMC symposium in November 2010.

Acknowledgements

A long process has finally reached its end – the work of several years has resulted in the completion of this thesis. The journey brought me all the way from Uppsala, Sweden to Leiden in the green heart of the Netherlands, via a memorable year in lively Amsterdam. Who would have guessed that a homebound person like myself would ever break up from a normal life in Sweden and move to a whole new country, with everything that accompanies such a decision? Considering the amount of hours that I have put into research, there are some obvious and important questions to be raised: Did fume hood life allow for the other parts of life to happen? Was the long journey worth it all? Looking back, the obvious answer to both questions is yes!

Work has certainly been an important part of my life these years, but in the long run it won't be only the research I will remember – but all the wonderful and talented people that I had the honor to meet and spend time with. The work described in this thesis was performed at the Biosyn group, Leiden University, and I am grateful to all the great chemists and scientists that have surrounded and inspired me. Although everyone contributed to the unique Biosyn-sphere there are a few people that deserve special gratitude: Caroline and Esther who guided me through the bureaucracy of university life, and anything that has to do with graduate studies and living in the Netherlands in general; Fons, Hans, Kees, Nico, and the AMAs, who were available for help regarding purification, analyses and various technical support; Leendert who introduced me to the lab; Pieter, Raúl and Stefan whom I had the pleasure to work with more closely during your master theses; all former and present members of the 'sugar-posse' were not only fun to work with but also granted many memorable moments outside of work; and the Monkey-lab, the most fantastic and fun working place I have ever had... It's five o'clock somewhere...

I wish to express a special word of gratitude to Boudewijn, Daniel, Marthe, NaRae, Pieter, and Wouter for proofreading this thesis. Any typos or errors are due to my own last minute changes.

Thanks also to all my former colleagues and great medicinal chemists who accompanied me in my pre-Netherlands life at the Department of Medicinal Chemistry in Uppsala. During my very first job in the Netherlands at the Academic Training Association, I had the pleasure to meet Ralph, Annelies and Thabo – I will never forget our sessions at the 'Big Fat Yellow Cat Café'! I also enjoyed spending time outside of work – thank you: Onne, Fenne, Edwin, Gert, Henk, Auk, Catherine, Guido, Marieke, John, Kristina H, Jorg, Kristina O, and Douwe.

Jag vill också innerligt tacka mina nära vänner Olle, Anders och Yogesh för att ha hållit vänskapen vid liv, trots de stora avstånden och tiden fylld med allehanda plikter. Jag hoppas på att vi får mer tid tillsammans framöver.

Familjen Nöteberg – Per, Elisabeth, Daniel, Rebecca, Christian och Katarina – tack för att ni välkomnat mig så varmt i er familj och för alla trevliga stunder tillsammans, var än i världen det må ha varit.

Morfar Evan: tack för alla härliga stunder ute på sjön och alla roliga historier, bättre morfar/kompis kunde man inte ha. Varma tankar går också till mormor Gertrud som inte finns med oss längre; en tuffare och starkare kämpe har jag aldrig sett maken till. Syster Kristina som jag är otroligt stolt över; världens bästa syster! (Hur var det nu, betalar du festen?) Mor Birgitta, utan dig skulle jag inte ha kommit så här långt i livet. Tack för allt du gjort och uppoffrat för att jag ska få ett bra och rikt liv.

Slutligen vill jag tillägna en kärlekshälsning till min underbara fru Anna. Med dig har jag redan fått uppleva så väldigt mycket härligt. Jag blir konstant överraskad, imponerad och förundrad över de egenskaper du besitter. Du ger mig inte bara lycka i form av dig själv, men du har också varit ett stort stöd under hela doktorandtiden. Med tanke på allt vi fått vara med om de här åren – och då främst födseln av Linnea och hennes kommande syskon (Esset m.fl.?!) – ser jag väldigt mycket fram emot vårt fortsatta liv tillsammans!

Leiden, March 2011