Internship Organic Chemistry
Final Report

Synthetic approach towards a panel of $^{13}$C$_5$-labeled glycosphingolipids for glycosphingolipid storage disorders

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Course
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Abstract
The aim of this project was to develop a synthetic approach towards a panel of $^{13}C_5$-labeled glycosphingolipids for glycosphingolipid storage disorders. The first step was to synthesize the sphingosine backbone with the emphasis on introducing the labeled fatty tail in the final step. The sphingosine backbone has been successfully synthesized from Fmoc-L-Serine. During the first step, Fmoc-L-Serine was converted to the corresponding Weinreb amide. After protecting the $1^\circ$ alcohol, the Weinreb amide was converted into a $\alpha$-$\beta$ ketone. This ketone was then selectively reduced to the anti-alcohol and afterward protected. After deprotection of the $1^\circ$ alcohol, the fatty chain was installed using cross metathesis. Previously, in work done by P. van Oerle, this happened after reducing the ketone. This new route allows for the installment of a fatty chain with $^{13}C_5$-atoms in the final phase, which is much more cost efficient.

The second step was the synthesis of four different sugar donors who would be coupled to the sphingosine backbone in a later stage. The four sugar donors of interest were galactose, glucose, lactose and 3-O-sulfogalactose. Galactose imidate and glucose imidate have successfully been synthesized and coupled to the sphingosine backbone. Deprotection of the protected galactosylsphingosine and glucosylsphingosine have been achieved as well and has been confirmed by LC-MS analysis. Preliminary NMR data suggests that lactose imidate has successfully been coupled to the sphingosine backbone. However, deprotection and more analysis are still necessary to fully identify this compound. The synthesis of the 3-O-sulfogalactose donor has not been finished yet by a lack of time and resources. At the moment, the synthesis is in its final stage and could be finished if more time was available.
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1 Introduction

1.1 Sphingosine
Sphingosine is the name of a group of amino alcohols that forms the basis skeleton for many lipids found in animal cells. The term sphingosine was first described in 1884 by J. L. W. Thudichum, but it took more than 60 years before the structure was finally elucidated as (2S,3R,4E)-2-aminooctadec-4-ene-1,3-diol by Herb Carter.\(^1\) (Figure 1)

![Figure 1: Molecular structure of a sphingosine.](image)

The structure of a sphingosine always contains a polar head, in this example the hydroxyl and amino groups, and a long apolar carbon chain. Lipids based on the sphingosine structure are called sphingolipids and can be found in the cell membranes of animal cells. The fatty tail sticks into the membrane, the polar head stays outside the membrane. They play a role in cell signaling, vesicular travel, and formation of specialized structures.\(^2\)

1.2 Ceramide

![Figure 2: Molecular structure of a ceramide.](image)

Ceramide is a term most often used for the collection of \(N\)-acylsphingosines. The consist out of a sphingosine backbone and a fatty acid tail. The \(N\)-fatty acid tails are 16 to 26 carbon atoms long and mostly saturated. Ceramides are also mainly found in cell membranes where they play a role in cell signaling. The particular type of cell signaling is determined by the moiety (sugars, phosphates or sulphates) attached to the ceramide. The ceramide that is shown in Figure 2 is the backbone of galactosylceramide, a ceramide that is attached to a galactose molecule.\(^2\) Eventually, enzymes in the human body, degrade glyceroceramides into individual building blocks; sugar, sphingosine, and a fatty tail. In some cases such an enzyme is defect. Such a defect leads, depending on which enzyme is defect, to a specific disease within a group of diseases; the lysosomal storage disorders.\(^2\)

1.3 Lysosomal storage disorders
Lysosomal storage disorders are a group of inherited metabolic diseases. Most of these disorders are caused by a deficiency in a single lysosomal enzyme. Its function is to degrade natural occurring compounds in the body. Interruption of this process by the deficiency of an enzyme results in the lysosomal accumulation of one, or many, natural compounds. This accumulation usually leads to severe health complications and eventually death. There are at least 47 known different lysosomal storage disorders and combined they appear once every 7700 births.\(^3,4\) The focus of this research turns towards a subgroup called sphingolipidoses. In this subgroup, the natural compounds that
accumulate are called glycosphingolipids. Figure 3 shows a schematic example of the enzymatic pathways that take place in the lysosomes.

The starting point of this enzymatic process is in the upper left corner with the galactosylceramide. In a normal functioning lysosome, galactose is cleaved off by β-galactosidase in the first step. Afterwards, ceramidase removes the N-palmitoyl fatty acid chain and a sphingosine remains. In patients with a glycosphingolipid storage disorder, the first step does not occur because the enzyme that is responsible for cleaving β-galactose is deficient. However, ceramidase is still active and will remove the N-palmitoyl fatty acid chain. This results in a glycosphingolipid that cannot be degraded any further, and therefore it accumulates in the lysosomes. Glycosphingolipids are therefore the primary focus of this research. If synthesized, they could be used as a diagnostic tool in mass spectrometry analysis (embedded with $^{13}$C$_5$-atoms) and many other research inquiries. Figure 4 shows an overview of the four target glycosphingolipids.\textsuperscript{5,6}

![Figure 3: Schematic overview of the pathways in a lysosome (Galactosylceramide as example).](image)

![Figure 4: Overview of target glycosphingolipids.](image)
1.4 Glycosphingolipid storage disorders
The lysosomal storage disorders were divided into five different groups; defects in glycan degradation, defects in lipid degradation, defects in protein degradation, defects in lysosomal transporters and defects in lysosomal trafficking. The glycosphingolipid storage disorders discussed in this subchapter are characterized by a defect in glycan degradation.

1.4.1 Globoid cell leukodystrophy (Krabbe disease)
Krabbe disease or globoid cell leukodystrophy is a lysosomal storage disorder which is caused by a deficiency of galactosylceramide-β-galactosidase. The onset usually starts between the age of 3 and 6 months. Most of the patients die before the age of two. The symptoms are mainly neurological because galactosylsphingosine (Figure 5) accumulates in the nervous cells that produce myelin. Myelin insulates and connects the nervous cell. Accumulation of galactosylsphingosine causes damage to the myelin and produces neurological symptoms. It deteriorates intellectual functions, motor skills and the ability to walk. The symptoms become progressively worse and usually lead to severe health complications and eventually death.

Figure 5: Molecular structure of galactosylsphingosine.

However, β-galactosidase is most likely not only involved in breaking down galactosylceramide. This becomes abundantly clear when looking at lactosylsphingosine (Figure 6).

Figure 6: Molecular structure of lactosylsphingosine.

Lactose is a disaccharide consisting out of β-galactose 1-4 linked to β-glucose. The first step in breaking down lactosylceramide is the removal of the galactose unit by β-galactosidase after which it becomes glucosylceramide. So far it hasn't been linked to a lysosomal storage disorder but the degradation of lactosylceramide might be affected by Krabbe disease.
1.4.2 Gaucher disease

Gaucher disease is a more prevalent lysosomal storage disorder that is inherited and characterized by a deficiency of β-glucosidase. This enzyme is responsible for the hydrolysis of the sugarsphingosine bond. If the enzyme is defect, glucosylceramide will not be broken down and glucosylsphingosine (Figure 7) will accumulate in the body.\(^\text{12}\)

![Figure 7: Molecular structure of glucosylsphingosine.](image)

The accumulation can happen in many different areas in the body. Gaucher disease is divided into different types which are described by the symptoms that occur in the patient. Gaucher disease can be roughly divided into non-neuropathic and neuropathic Gaucher disease. Non-neuropathic Gaucher disease doesn’t affect the central nervous systems and can lead to the enlargement of the liver and spleen, anemia and bone abnormalities. Neurono pathic Gaucher disease affects the central nervous system and leads, besides all the symptoms described above, also to brain damage. It is therefore more lethal than non-neuropathic Gaucher disease.\(^\text{12}\)

1.4.3 Metachromatic leukodystrophy

Metachromatic leukodystrophy is a lysosomal storage disorder that is characterized by a deficiency of arylsulfatase A. Arylsulfatase A is an enzyme that degrades sulfatides by removing the sulphate group. 3-O-Sulphogalactosylsphingosine, (Figure 8) or sulfatide, accumulates in the nervous cells that produce myelin.\(^\text{13,14}\)

![Figure 8: Molecular structure of 3-O-sulphogalactosylsphingosine.](image)

Accumulation of 3-O-Sulphogalactosylsphingosine in the nervous cells causes the destruction of white matter. The degradation of white matter causes all kind of neurological problems; loss of the ability to walk, incontinence, loss of sensation in the extremities and hearing loss.\(^\text{13,14}\)

1.4.4 Treating lysosomal storage disorders

In general, the symptoms of a lysosomal storage disorder are caused by the unwanted accumulation of natural compounds in the lysosomes. The lysosomes fulfill an important role in the human body; they degrade oligosaccharides and complex fatty acid molecules. The accumulation of individual compounds reduces the metabolic function of the lysosomes and eventually they will die. At the moment there are three major ways to treat lysosomal storage disorders; enzyme replacement therapy (ERT), adding chaperones (CMT) and substrate reducing therapy (SRT).

Enzyme replacement therapy reduces the build up in the lysosomes by injecting the active enzyme into the body. This will revert the build up and decreases the toxicity to the lysosomes. It relieves the symptoms.

Substrate reducing therapy inhibits the biosynthesis of the substrate in an earlier stage and so reduces the build up and therefore the symptoms.
The last strategy is to introduce chaperones into the system. They will coordinate with the enzyme to reactivate the enzyme.\(^4\)

### 1.4.5 \(^{13}\)C enriched diagnostic tools

Even though it is possible to treat lysosomal storage disorders, it is not yet possible to diagnose them. The reason for this is that there are no available standards to use as a comparison in an analytical assay. In the process of analyzing a human sample, a certain amount is always lost to the assay. This could give a false positive result. There is no way of knowing what the actual concentration was before the assay. That’s where a \(^{13}\)C enriched standard comes in. If a \(^{13}\)C enriched standard of the target compound is available, the extraction can be spiked with an exact amount of this standard. LCMS analysis will produce a spectrum similar to the one in Figure 9. This spectrum is the results of recent work that was done by Gold, H. et all\(^{15}\) and shows two main compounds. LysoGb3 and \(^{13}\)C\(_5\)-lysoGb3. At this point, the ratio between the areas of the peaks can be used to calculate the correct amount of lysoGb3 in the assay.\(^{15,16}\)

![Figure 9: Example of mass analysis with \(^{13}\)C\(_5\)-enriched compounds.](image)

### 1.5 The aim of this project

The goal of this project was to develop a synthetic approach towards a panel of \(^{13}\)C\(_5\)-labeled glycosphingolipids for glycosphingolipid storage disorders. This primary goal was divided into a few sub-goals. First, the optimization of the synthesis of the sphingosine backbone with the primary focus on the introduction of the fatty tail in the final step. The \(^{13}\)C\(_5\)-atoms would be incorporated into the fatty tail. Therefore it would be more economically favorable to introduce this in the last stage. Second, the synthesis of the individual sugar donors with an imidate group on the anomeric position. Galactose, lactose, and glucose will be fully benzoylated except for the anomeric position, which will have an imidate as a functional group. 3-O-sulphogalactose will have a benzylidene bridge on the four and six position, a protected sulfate group on the three position and a benzoyl on the two position.

The final goal was to couple these four sugar donors to the sphingosine acceptor and remove all the protecting groups.
2 Results and discussion

2.1 Retrosynthetic approach towards galactosylsphingosine

Figure 10 gives a short overview of the synthetical route that has been used for the synthesis of galactosylsphingosine. The synthetical routes for the synthesis of glucosyl- and lactosylsphingosine are identical to the route described below. The route for the synthesis of 3-O-sulfugalactose is slightly different and will be discussed in chapter 2.4.1.

![Diagram of retrosynthetic approach towards galactosylsphingosine](image)

The retrosynthesis of the sphingosine backbone is based on the work done by Yamamoto\textsuperscript{17}, with a slightly different approach to protecting the intermediates. The first notable difference is the use of the Fmoc protecting group instead of the t-Boc (tert-butylcarbonyl) group. Fmoc stands for fluorenylmethyloxycarbonyl and is a well known protecting group for amines.\textsuperscript{18} They have a different stability. The t-Boc group is stable to basic conditions and removed under strong acidic conditions.
The Fmoc group is stable to acidic conditions and removed under basic conditions. This change allows for the use of slightly acidic conditions throughout the process.

The first intermediate, Fmoc-L-Serine is available commercially and was purchased. The carboxylic acid group was then converted into a Weinreb amide using carbodiimide chemistry. The primary alcohol was protected using tert-butyldimethylsilyl chloride. Next, a double bond was introduced which yielded a α-β unsaturated ketone. This ketone function was selectively reduced to an alcohol and protected with a para-methoxybenzyl group. After a selective deprotection of the silyl ether, a cross metathesis reaction was used to introduce the fatty chain.

The retrosynthesis of the galactose donor was based on previous work done by P. van Oerle. In the first step D-(+)-galactose was fully protected with benzoyl groups after which the anomeric position was deprotected. In the last step, an imidate was placed on the anomeric position. The coupling of the galactose donor to the sphingosine acceptor is based on the work done by P. Wisse. The donor and acceptor have been coupled using a glycosylation reaction.

All of the reactions will be elaborated in the next subchapters.

### 2.2 Synthesis of the sphingosine backbone

#### 2.2.1 Synthesis of the Weinreb amide

The first step is to change the reactivity of the carbonyl function towards a higher susceptibility for a nucleophilic attack. This is achieved by converting the carboxylic acid group into a Weinreb amide and can be seen in Figure 11.

Figure 11: Mechanism for the Weinreb amide synthesis.
The mechanism is based on a Steglich esterification. First, the carboxyl group is converted into an activated ester with \(N\)-(3-Dimethylaminopropyl)-\(N'\)-ethylcarbodiimide hydrochloride (EDC.HCl). It is activated because every nucleophilic attack leads to the expulsion of a very stable urea group. This drives the reaction to the right and makes sure that almost all of the EDC.HCl is replaced by Hydroxybenzotriazole (HOBt). This intermediate is a lot more stable and is easily replaced by \(N,O\)-dimethyl hydroxylamine hydrochloride to form the final product. Most of the literature describes this reaction or a similar reaction with either EDC.HCl or HOBt. Both perform a similar role by activating the carbonyl. However, with only EDC.HCl the yield was 44% after purification by column. Earlier work of P. van Oerle (Bio-Organic Synthesis) indicated a yield of 75% with both present and only purification after the second step. The second step, of which the mechanism can be seen in Figure 12, was the protection of the primary alcohol with tert-butyldimethylsilyl chloride (TBDMS-Cl).

![Figure 12: protecting the primary alcohol with TBDMS-Cl](image)

After the new addition of HOBt the resulting crude reaction mixture was used in the second step. After purification by column, this indeed yielded the protected Weinreb amide with a yield of 75%. In the NMR spectrum of the purified compound (Appendix A2) the newly formed Weinreb amide can be observed by the two singlets at 3.75 and 3.23 ppm which correspond respectively to an \(N\)-methyl and \(O\)-methyl group. The TBDMS group can be seen around 0.8 ppm (t-butyl group) and directly next to the TMS peak (2x methyl).
2.2.2 The Grignard reaction

After installment of the Weinreb amide, the carbonyl is now more susceptible to a nucleophilic attack by a Grignard reagent. This opens up the possibility to introduce a α-β unsaturated ketone. The mechanism of this reaction can be seen in Figure 13.

![Mechanism of the Grignard reaction](image)

The first step that is shown in the mechanism is the same as a standard Grignard reaction. The nucleophilic attack of vinylmagnesiumbromide results in the tetrahedral intermediate 3a which is further stabilized by the methoxy group. From this point, there are two possible outcomes which are determined by the work up procedure that is used next. Yamamoto\textsuperscript{17} specifically mentioned that the reaction mixture had to be quenched by pouring it slowly into an ice cold 2M HCl solution followed by a quick extraction with EtOAc. This might seem odd at first but has an absolutely logical explanation which was explained by previous work done in the lab by P. Wisse and M. de Geus.\textsuperscript{22} When slowly poured into an ice cold 2M HCl solution the desired product 4 will be formed in 67%, and the free Weinreb amide will be immediately protonated which prevents it from reacting any further. In the second case, slightly acidic water was added to the reaction mixture. At first the
reaction proceeds as normal, the acidic conditions free the Weinreb amide, and product 4 is formed. However, there is not enough acid to immediately protonate the free Weinreb amide. It still has a lone pair that can participate in a 1,4-addition. This is called a Michael addition and will yield the β-aminoketone 4a up to 70%.\textsuperscript{22,23} Quickly extracting the reaction mixture is necessary for both steps to prevent the deprotection of the silyl ether.

The NMR analysis (Appendix A2) of compound 4 shows two new peaks in the region between 5.5-7 ppm. They correspond to the two newly formed double bond protons.

2.2.3 Stereoselective reduction of an α-β unsaturated ketone

After installment of the double bond, the ketone group needs to be reduced to an allylic alcohol with the right stereochemistry. In the original paper by Yamamoto\textsuperscript{17}, it proved to be difficult to reduce the ketone to the allylic alcohol with the right stereochemistry. They tried a variety of reagents before using the method described by Toshikazu.\textsuperscript{24} This method uses a sterically hindered reducing agent and limits the possible configurations of the starting material by cooling the reaction mixture to -78°C. (Figure 14)

![Figure 14: Mechanism of the selective reduction.](Image)

To understand the reaction the mechanism is drawn with Newman projections. Once cooled to -78°C chelation with Lithium takes place. This forces the molecule in a conformation where only the back end of the molecule is available to attack by a bulky reducing agent. This yields the anti-product 5 in 73% after purification by column.\textsuperscript{24,25} Compound 5 was characterized by NMR (Appendix A3) and compared to the data available. Around 4.3 ppm appears a new proton (attached to C3), clearly visible between the two peaks belonging to the Fmoc group. It was assumed to be the pure anti-product because previous work done by Yamamoto\textsuperscript{17} optimized these conditions and they were already tested in the lab by my predecessor.
2.2.4 Protecting the allylic alcohol with the para-methoxybenzyl group

Protection of the secondary alcohol could not be done under the standard conditions. Typically a para-methoxybenzyl group is introduced with a strong base. A strong base will remove the Fmoc group. So, in our case, was chosen to protect the secondary alcohol with para-methoxybenzyl trichloroacetimidate (PMB-TCA). It can be used to protect under mildly acidic conditions using Scandium(III)triflate.\(^\text{26}\) (Figure 15)

![Reaction Mechanism](image)

Figure 15: Mechanism for benzyl protecting.

After activation by Scandium(III)triflate the imidate part is expelled as trichloroethaneamine, which leaves a positive charge on the oxygen atom. Delocalization stabilizes this charge. To simplify not all the resonance structures are drawn. Now the electrophilic benzyl position is can be attacked by a soft nucleophile like the secondary alcohol. After proton transfer and purification by column compound 6 can be isolated with a yield of 50%. However, NMR data showed that the product was still impure. On TLC the product consists of one spot, but the NMR data suggest two PMB groups. Appendix A4 contains the spectra of compound 6, as it was obtained in its purest form after the optimization of this protocol. There are two peaks that are easily identifiable and characteristic for a PMB group, the doublet around 6.8 ppm, belonging to two aromatic protons, and the singlet around 3.8 ppm, which belongs to the CH\(_3\) group. Both of these appeared to be double in the previous experiment. Most likely it is still some PMB alcohol that is trapped between the molecules of the product. This also indicates that some of the PMB reagent is hydrolyzed during the reaction, and this might be an explanation for the low yield. Some literature research revealed two other possibilities that could improve the yield. The use of a more stable PMB-imidate reagent; \(p\)-Methoxybenzyl-N-
phenyl-2,2,2-trifluoroacetimidate (PMB-TFA)\textsuperscript{27} and the use of a different catalyst; 10-camphorsulphonic acid\textsuperscript{28}, which is a protic acid and slightly stronger than the previously used Lewis acid. The obtained data can be seen in Table 1.

Table 1: Overview of the data obtained from the PMB protection optimization.

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<tr>
<th>Catalyst</th>
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<th>Solvent</th>
<th>Yield (%)*</th>
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<td>Scandium(III)triflate</td>
<td>TCA</td>
<td>DCM</td>
<td>50</td>
<td>Impure</td>
</tr>
<tr>
<td></td>
<td>TFA</td>
<td>DCM</td>
<td>57</td>
<td>Pure</td>
</tr>
<tr>
<td>10-Camphorsulphonic acid</td>
<td>TCA</td>
<td>Tol</td>
<td>79</td>
<td>Impure</td>
</tr>
<tr>
<td></td>
<td>TFA</td>
<td>Tol</td>
<td>58</td>
<td>Pure</td>
</tr>
</tbody>
</table>

\*Yield is based on weight, and says nothing about purity. Most of the reactions gave inseparable mixtures. The yields in this table should not be seen as an absolute numbers but as an indication to how much of the reaction mixture was isolated.

The initial reaction with TCA was performed in DCM but according to Barroca-Aubry\textsuperscript{27} toluene would give a higher yield if TFA was used to protect a secondary alcohol. To see the difference, the reactions were performed in both DCM and toluene. This change gave comparable results. However, something else became apparent through these experiments. When the reaction was performed with TFA instead of TCA, the final product could be purified easily by column. The excess reagent is now visible on TLC and therefore can be separated from the product.

The change to 10-camphorsulphonic acid was of interest because Wang used TCA to protect an allylic secondary alcohol, which corresponds to the system used during this research. However, the rest of the molecule different. (Figure 16)

![Figure 16: similarity between Wang's compound and compound 5](image)

The use of a new catalyst improved the reaction slightly but not in a spectacular way. The yield was still only 58%. Wang's protection\textsuperscript{28} gave a yield of 93%. There is no immediate explanation for this. By lack of more time, it was decided that this was good enough for now.
2.2.5 Selective deprotection of TBDMS
Deprotection of a silyl ether is a relatively simple process and can be seen in Figure 17.

Fluoride has a particular fondness for silicium, as soon as both are together in solution they will connect which effectively separates the silyl group from the alcohol. Tetrabutylammonium fluoride (TBAF) was used as a fluoride source. However, TBAF is very basic, and can destroy the Fmoc group in the process. To prevent this, 4 eq of acetic acid were added before adding the TBAF. This reaction gave compound 7 with a yield of 94%. Appendix A5 contains a $^1$H spectrum of compound 7. However, in the end, the available $^{13}$C spectrum didn't have the necessary intensity and couldn't be included on time.

2.2.6 Cross metathesis
Alkene metathesis is a relatively new organometallic technique that was developed by Robert Grubbs, Yves Chauvin and Richard Schrock, which awarded them the Nobel Prize in Chemistry in 2005. Cross metathesis is just one of several classes of metathesis and can be described as a reaction were two alkenes switch from functional groups. (Figure 18)

In this case olefin metathesis is a very useful reaction. It was used to install the tail of the sphingosine backbone. Cross metathesis uses an organometallic complex depicted as M in Figure 18. Figure 19 shows the entire catalytic cycle.
Figure 19: catalytic cycle of olefin metathesis
The catalyst depicted in the upper left corner is called Grubbs 2nd generation catalyst. The first step in the mechanism is the loss of the phosphine ligand. This opens up the space necessary for the substrate to enter the catalytic cycle. When the substrate enters the catalytic cycle, it undergoes a [2+2] cycloaddition with a metallacyclobutane structure as a result. This is reversed in the next step but with a slight difference, styrene is expelled, and the substrate stays with the catalyst. This process can always go either way, but for informative purposes, only the favorable option is drawn. The next steps can also go either way, a second substrate can enter the cycle, the same substrate can enter the cycle or the starting can enter the cycle. There are some rules about what is more favorable, but that will be explained at a later stage. Let's assume that a second substrate enters the catalytic cycle. After the [2+2] cycloaddition and the reverse, there is now a new compound with functional groups from substrate 1 and 2. Ethene stays with catalyst and can be replaced in the same fashion with styrene. That concludes the catalytic cycle and also drives the reaction to the right because of the loss of ethene.\textsuperscript{16,30-31}

As mentioned earlier, the reaction can go at each stage either way. Besides that, it does not always react in a desirable way. In a lot of cases, two molecules of the same substrate make dimers instead of reacting with a second substrate. In 2003, Chatterjee\textsuperscript{32} published an article with a table that predicts the success of different metathesis based on the substrates ability to form homodimers over the ability to form heterodimers. Four basic types can be seen in Table 2.\textsuperscript{32}

Table 2: Alkene categorization for cross metathesis.

<table>
<thead>
<tr>
<th>Type</th>
<th>Reaction Type</th>
<th>Substrate Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>fast homodimerization</td>
<td>Terminal alkenes, 1\textsuperscript{0} allylic alcohols, esters, allyl boronate esters, allyl halides, styrenes (without large ortho substituent), allyl phosphonates, allyl silanes, allyl phosphine oxides, allyl sulfides, protected allyl amines</td>
</tr>
<tr>
<td>Type II</td>
<td>slow homodimerization</td>
<td>Styrenes (large ortho substituent), acrylates, acrylamides, acrylic acid, acrolein, vinyl ketones, unprotected 3\textsuperscript{0} allylic alcohols, vinyl epoxides, 2\textsuperscript{0} allylic alcohols, perfluorinated alkane olefins</td>
</tr>
<tr>
<td>Type III</td>
<td>no homodimerization</td>
<td>1,1-disubstituted alkenes, non-bulky trisubstituted alkenes, vinyl phosphonates, phenyl vinyl sulfone, 4\textsuperscript{0} allylic carbons (all alkyl substituents), protected 3\textsuperscript{0} allylic alcohols</td>
</tr>
<tr>
<td>Type IV</td>
<td>spectators to cross metathesis</td>
<td>Vinyl nitro olefins, protected trisubstituted allyl alcohols</td>
</tr>
</tbody>
</table>

Simply put, Type I alkenes are in general electron rich and unhindered, moving down the line the alkenes become increasingly electron poor and more hindered. The best possible reaction would go between a Type I alkene and a Type II alkene. Type 3 alkenes can react with Type I and II but at a slower rate. Type IV alkenes show no reactivity towards the catalyst.\textsuperscript{33,32}

As mentioned earlier, it is important that the metathesis is the final, or at least as close as possible, to the last reaction. Therefore, compound 5, 6 and 7 have all been tested for their relative reactivity. The results can be seen in Table 3.
Table 3: Reactivity towards cross metathesis for compound 5, 6 and 7

<table>
<thead>
<tr>
<th>Entry</th>
<th>R1</th>
<th>R2</th>
<th>Type</th>
<th>Grubbs 2nd gen (mol%)</th>
<th>Solvent</th>
<th>Heating (°C)</th>
<th>Acetic acid (eq)</th>
<th>CuI (mol%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>TBDMS</td>
<td>II</td>
<td>10</td>
<td>DCM</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>TBDMS</td>
<td>II</td>
<td>10</td>
<td>Et₂O</td>
<td>40</td>
<td>0</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>TBDMS</td>
<td>II</td>
<td>10</td>
<td>Tol</td>
<td>40</td>
<td>0</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>PMB</td>
<td>TBDMS</td>
<td>III</td>
<td>10</td>
<td>DCM</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>PMB</td>
<td>TBDMS</td>
<td>III</td>
<td>10</td>
<td>Et₂O</td>
<td>40</td>
<td>0</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>PMB</td>
<td>TBDMS</td>
<td>III</td>
<td>10</td>
<td>Tol</td>
<td>40</td>
<td>0</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>7</td>
<td>PMB</td>
<td>H</td>
<td>III</td>
<td>10</td>
<td>DCM</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>8</td>
<td>PMB</td>
<td>H</td>
<td>III</td>
<td>10</td>
<td>Et₂O</td>
<td>40</td>
<td>0</td>
<td>30</td>
<td>/</td>
</tr>
<tr>
<td>9</td>
<td>PMB</td>
<td>H</td>
<td>III</td>
<td>10</td>
<td>Tol</td>
<td>40</td>
<td>0</td>
<td>30</td>
<td>17</td>
</tr>
</tbody>
</table>

The first entry was expected to perform reasonable well. 1-Pentadecene is a terminal alkene which categorizes it as Type I. Compound 5 is an unprotected allylic alcohol and therefore a Type II alkene. It is expected that this combination reacts well. A yield of 62% is not phenomenal, but it is the same yield that P. van Oerle obtained. The addition of acetic acid needs some further elaboration. Previous work done in the lab by P. Wisse showed that the double bond sometimes shifts during the reaction. Grubbs published an article with a list of substances that prevent this from happening. Acetic acid prevents the double bond from moving although it is not yet clear why.

Entry two and three need some further elaboration as well. Previous work done by M.A.R. de Geus showed that the addition of Cu(I)I increased the reactivity of the Grubbs 2nd generation catalyst. He proposed a mechanism where iodine replaces chlorine on the catalyst. Iodine is bigger than chlorine and might push the phosphine ligand out of place. This would increase the reactivity. Therefore, his method was tried for all compounds. It had to be done in both Et₂O and toluene because compound 7 does not dissolve in Et₂O. For entry one to three the yield is not increased by the addition of Cu(I)I. It might either not work for this substrate or the lack of acetic acid yields more side products. However, this is just speculation and was not investigated.

The fourth entry was expected to perform less than the first three. The allylic alcohol is now protected which makes it a Type III alkene and according to the predictions, it will react slower with a Type I alkene than a Type II. The results matched the expectations. The overall yields are lower. However, an unexpected result came up. The yield increases from entry four up to entry six. This indicates that Cu(I)I might have a positive effect on this particular synthesis. It also suggests that the chosen solvents make a difference. However, because this was not the desired synthesis, it was not pursued further.

The last entry, seven to nine, were not expected to perform any better than entries four to six because he predictions by Chatterjee only mention the first three to four carbon atoms. The change happens at the other end of the molecule which is more than four carbon atoms away. However, entry 7 performs miraculously well, even better than the first entry. At this moment, there is no logical explanation for this result, but it is precisely the result that was desired. NMR spectra of
compound 8 can be found in Appendix A6. Most of the protons from the freshly installed fatty chain give a signal around 1.25 ppm except for the protons which are right next to the alkene (2.1 ppm) and the terminal protons along the chain (0.8 ppm).

2.3 Synthesis of the galactose donor

2.3.1 Benzylation of galactose

The galactose donor had to be designed in a way that would leave room for soft deprotection methods. Therefore was decided to use benzyol groups as protecting groups. They can be easily removed using low concentrations of NaOH in MeOH. The benzylation of galactose, Figure 20, is in theory, an acylation reaction.

\[
\begin{align*}
\text{R} &= \text{hydroxyl} \quad \rightarrow \\
\text{O} & \quad \text{OH} \quad \rightarrow \\
\text{O} & \quad \text{R} \quad \text{O}^- \\
\text{N} & \quad \text{Cl} \\
\text{O} & \quad \text{Cl} \\
\end{align*}
\]

Figure 20: Mechanism of the benzylation of galactose.

In the first step, the hydroxyl group is deprotonated by pyridine. Oxygen now becomes a negatively charged species and can perform a nucleophilic attack on the electrophilic carbon from benzyol chloride. This yields a tetrahedron after which the most stable leaving group is expelled, chlorine. This reaction yielded fully benzyolated galactose 10 in quantitative yield.\textsuperscript{21} Both \textsuperscript{1}H and \textsuperscript{13}C spectra of compound 10 are included in Appendix A7. At this phase the product is fully β orientated. Full peak assignment can be found in the experimental section.
2.3.2 Selective deprotection of the anomeric position

The galactose donor has an imidate at the anomeric position in the final stage, however, first the anomeric position needs to be selectively deprotected. This can be achieved with hydrazine acetate.

The mechanism can be seen in Figure 21.

Before a lone pair of nitrogen can attack the electrophilic carbonyl carbon it needs to be more electrophilic. Two things contribute to this first step; the carbonyl oxygen is protonated, and the oxygen in the sugar molecule acts as an electron withdrawing group. The latter is also the explanation for the fact that this reaction only takes place at the anomeric position. After activation of the carbonyl group, a lone pair of nitrogen can attack the electrophilic carbon atom and form a tetrahedral structure. Next a proton transfer from hydrazine to the ether oxygen and deprotonation of the other oxygen results in the expulsion of selectively deprotected galactose 11 in neutral form.35

Both $^1$H and $^{13}$C spectra of compound 11 are included in Appendix A8. Deprotection of the anomeric position yields a α-β mixture. This can be seen clearly in the $^1$H spectrum, it seems to be a mixture of two compounds. Important to notice is the shift of the anomeric proton, which is a clear indication that the anomeric position has changed. It shifts from 6.97 ppm in compound 10, to around 6.10 ppm in compound 11.
2.3.3 Synthesis of an imidate

The final step is the installment of an imidate at the anomeric position. An imidate is a strongly electron withdrawing group and therefore a good leaving group. This makes it suitable for glycosylation. The mechanism of this reaction is schematically presented in Figure 22.

The anomeric alcohol is readily deprotonated by potassium carbonate to form a good nucleophile. The negatively charged oxygen species can then attack the electropositive carbon atom adjacent to the nitrogen in trichloroacetonitrile which yields an imine after protonation. This yields the galactose imidate donor 12. Compound 12 has been analyzed by NMR after every step. However, the first two steps are straightforward and well documented. Therefore the spectra are not included in this report. The spectrum of compound 12 can be found in Appendix A9. An imidate is characterized by a singlet around 8-9 ppm, which in this is clearly visible around 8.6 ppm. This peak was not present in the previous step and therefore it is an excellent indication that the right product has formed. The anomeric positions shifts as well, from around 6.10 ppm in compound 11, to 6.91 ppm in compound 12. The $^1H$ spectrum still shows a slight amount of $\alpha$ product. However, most of the product is $\beta$-orientated.
2.4 Synthesis the 3-O-sulfogalactose donor

2.4.1 Retro-synthesis
The retrosynthetic approach in Figure 23 shows the proposed synthetic pathway to synthesize the 3-O-sulfogalactose donor 20.

![Chemical diagram of the synthesis process]

The first step is the replacement of the anomeric acetyl group with thiophenol. Next, all the remaining acetyl groups are removed after which a benzylidene bridge is formed. Then the protected sulfate group is selectively introduced at the 3-O position. The last free hydroxyl group is protected with a benzoyl group. In the final two steps, thiophenol is first hydrolyzed after which it is replaced by an imidate.

Figure 23: Retrosynthetic approach for the synthesis of the 3-O-sulfogalactose donor.
2.4.2 Replacing the anomeric acetyl with thiophenol

Thioglycosides are important intermediates for many synthetic pathways to more complex biological molecules. They are stable, prevent α-β mixtures, and it's possible to use them in glycosylation reactions. In this case, it allows for an easy installment of a trichloroacetimidate group. Replacing the anomeric acetyl group with thiophenol is a glycosylation reaction. Glycosylation is the coupling of a sugar donor to a hydroxyl group (acceptor), or another functional group. A simple schematic overview of the mechanism is presented in Figure 24.36

In the first step the leaving group leaves after activation. This yields a reactive intermediate that is referred to as the oxocarbenium ion. This reactive species has not yet been confirmed but is expected to be the major contributing species to this reaction. Next, a nucleophile can attack and the donor is coupled to the acceptor. The new configuration depends on the neighboring groups. Alpha selectivity is primarily achieved due to a constructive overlap in axial conformation between the orbitals of the anomeric oxygen and the ring oxygen. However, esters give high β selectivity. This is achieved by neighboring group effect.36 (Figure 25)

After activation, the oxocarbenium ion is stabilized by the formation of an acetal. This acetal blocks any approach from the bottom and therefore hinders the formation of α products and ensures β selectivity. Figure 26 combines these notions and shows an overview of the mechanism for this reaction.
This reaction only takes place at the anomeric position because of the electron withdrawing effects of the ring oxygen. The anomeric position is the only position that is influenced by this and therefore is special. This reaction was performed without any problems and gave after purification with silica gel chromatography quantitative yield of compound 14. Appendix A10 contains the $^1$H and $^{13}$C spectra for compound 14. Two new multiplets are visible in the aromatic area, with an area of 5. This indicates the presence of an aromatic ring.

### 2.4.3 Transesterification: deacetylation reaction

Removing the remaining acetyl groups is a base catalyzed transesterification reaction. The mechanism can be seen in Figure 28.

The sodium methoxide anion attacks the carbonyl carbon, and a tetrahedral structure is formed. After protonation of the initial ester oxygen by methanol the tetrahedral structure collapses and methyl ethanoate is formed together with another methoxide anion. This makes the methoxide anion a catalyst and therefore only a few drops of 30% NaOMe/MeOH are sufficient to drive this reaction to full completion. The reaction repeats itself until all acetyl groups are removed. This reaction was carried out without any problems and gave compound 15 with a yield of 95%. Appendix A11 contains the $^1$H and $^{13}$C spectra of compound 15. It is clearly visible that the acetyl peaks, previously between 1.97-2.10 ppm, are now gone.
2.4.4 Synthesis of a benzylidene acetal

In the next step both the 4-O and 6-O positions are protected by creating an acetal. A schematic overview of this mechanism can be seen in Figure 28.

![Figure 28: Mechanism of building the benzylidene bridge.]

In the first step, one of the methoxy groups is protonated by \( p \)-toluenesulfonic acid. This places a positive charge on the oxygen atom and reduces the electron density at the carbon between the two methoxy groups. From here on there are two possibilities; a direct attack on the now more electrophilic carbon atom, or first the expulsion of methanol followed by the same attack. Personally, I find the latter the best option because the charge is divided over both the oxygen and the carbon atom, making it more stable. After the attack of the hydroxyl on the 6-O position, a hydrogen transfer will take place. It can move directly towards the second methoxy group, or it can be transferred through \( p \)-toluenesulfonic acid. Now the reaction repeats itself but this time with the hydroxyl group at the 4-O position. After deprotonation, this will result in compound 16. Normally, this reaction is a reversible reaction. In our case this is prevented by removing the methanol under reduced pressure in a rotary evaporator during the reaction. This reaction gave compound 16 with a yield of 84%\(^{38}\). Appendix A12 shows the \(^1\)H-NMR spectrum of compound 16. It shows a singlet at 5.48 ppm which is characteristic for extra proton from the benzylidene bridge.

2.4.5 Introducing the protected sulfate group at the 3-O position

Introducing a sulfate group onto the 3-O position has been done before at a later stage; after the coupling of galactose with the sphingosine backbone.\(^{14}\) However, an already sulfated galactose donor has not been coupled before to a sphingosine backbone. The reaction itself, however, has been performed.\(^{39}\) The protected sulfate group has been synthesized according to Desoky et al. and introduced in an early stage of the building block. The mechanism of this reaction can be seen in Figure 29.\(^{39}\)
The hydroxyl group on the 3-\(O\) position attacks the sulfur center as a soft nucleophile and 1,2-imidazolium triflate leaves with a negative charge on the nitrogen. This is possible because the anion forms a conjugated aromatic system together with the two \(\pi\)-bonds stabilizing the charge. In the final step, DMI abstracts the proton to form compound 17. NMR analysis of this compound (Appendix A13) shows two protons at 4.66 and 4.85 ppm that correspond to the two protons in trichloroethanol. They do not couple with any of the sugar protons in a COSY spectrum. However, to be sure our spectra were checked to the available spectral data. As mentioned before the regioselectively highly depends on the substrate and the conditions used for introducing the protected sulfate group. According to their data it was important that DMI was added separately and slowly over a few hours to a solution of 16 and 17a. Other combinations lead to either the 2-sulfated or 2,3-disulfated products. These kind of mixtures are tough to separate and preferably avoided. This reaction was except for one variable accurately performed according to the experimental data in the article but our yield was only 15% compared to 88%. The only variable that is not thoroughly described in the article is the rate at which the base is added. During our experiment, a sixth of the total amount of base was added slowly every hour. This might have been too fast. They mention the use of a syringe pump but not the rate of the addition. Therefore it seems to be a difficult reaction to master. By a lack of time no more experiments have been performed to improve the yield of this reaction. However, we still have ideas to improve this reaction given enough time to perform them. The base should be added with a syringe pump, and the reaction mixture should be analyzed every hour with LCMS. This might give an clue for an optimal rate of addition.

### 2.4.6 Benzoylation of the 2-\(O\) position

The last hydroxyl group, on the 2-\(O\) position, has been protected with a benzoyl group. A benzoyl group allows for a soft basic introduction and easy basic removal together with the Fmoc group from the sphingosine backbone. The overall reaction scheme can be seen in Figure 31.
The mechanism of this reaction has been thoroughly discussed in chapter 2.2.2 and therefore will be not be discussed here. This reaction was performed with a yield of 55%. This yield seems a bit on the low side considering it is supposed to be a very easy reaction. However, this reaction has been performed twice so far and both times gave the same yield. By a lack of time, this reaction has not been optimized yet. Characterization of this reaction has been performed with NMR. Appendix A14 contains $^1$H and $^{13}$C spectra of compound 18. Compound 17 and 18 only differ in one benzoyl group. This is visible in the aromatic region but can't be relied upon for full characterization. However, this compound has been thoroughly analyzed with COSY and HSQC.

### 2.4.7 Hydrolysis of the thiophenol group

As mentioned earlier, in subchapter 2.4.2, the thiophenol group is an important intermediate because it protects the anomic position until the final stage when the trichloroacetimidate group is placed at the anomic position. Hydrolyzing the thiophenol group is usually achieved with either NIS or NBS in a wet organic solvent. However, because the benzylidene is acid labile a water free method is desired. Such a method was developed in-house by Ana R. de Jong et. al. A schematic overview of the proposed mechanism can be seen in Figure 30.

Figure 30: Hydrolysis of the thiophenol group (mechanism).
Sulfur is attracted by iodine and becomes positively charged. The thiophenol group now has become a good leaving group, and after an oxocarbenium ion is formed, TFA can attack the anomeric position which yields the intermediate 18a. The existence of this intermediate has been proven by the team when they followed this reaction with NMR. After addition of piperidine the ester is hydrolyzed and a α/β mixture of 19 is obtained. This reaction gave a yield of 90%. Appendix A15 contains the 1H and 13C spectra for compound 19. The anomeric proton shifts from 4.87 ppm in compound 18, to around 5.7 in compound 19.

2.4.8 Synthesis of an imidate
The last and final step in the synthesis of this building block is the installment of a trichloroacetimidate group on the anomeric position. So far this has not been achieved yet. The first experiment was probably too basic and gave a different product of which the nature still is unclear. By a lack of time and compound from the previous step, it was not performed again. However, we are confident that reducing the basicity and using the conditions described in this article will yield our desired product 20.

2.5 Synthesis of the glucose and lactose donors
The synthesis of the glucose 21 and lactose 22 donors are similar to the synthesis of the galactose donor. Both the glucose and lactose donor were already synthesized previously by a colleague. Before using them in the coupling reactions, they have been purified again with silica gel chromatography and thoroughly checked by NMR.
2.6 Coupling the sugar donors to the sphingosine acceptor

General glycosylation reactions are discussed in chapter 2.4.2. The coupling of a sugar donor to the sphingosine backbone follows the same rules explained in that subchapter. Figure 31 combines these basic notions and shows the proposed mechanism for this reaction.

![Mechanism of the glycosylation reaction](image)

Figure 31: Mechanism of the glycosylation reaction.

After coordination with TMSOTf (Lewis acid), the imidate is expelled as an amide. The resulting oxocarbenium ion is stabilized by the neighboring group effect. This ensures that the sphingosine acceptor can only attack from the side to give β coupled glycosylsphingosine.

This reaction was optimized using galactose 12 as donor. Table 4 shows an overview of the conditions used. All reactions were purified with silica gel chromatography and size exclusion chromatography.

<table>
<thead>
<tr>
<th>Donor (eq)</th>
<th>Acceptor (eq)</th>
<th>Catalyst</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>BF$_3$·Et$_2$O (1.4 eq)</td>
<td>/</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>BF$_3$·Et$_2$O (0.2 eq)</td>
<td>/</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>TMSOTf (0.2 eq)</td>
<td>67</td>
</tr>
</tbody>
</table>

Table 4: reaction conditions optimizing glycosylation

High amounts (1.4 eq) of BF$_3$·Et$_2$O resulted in a mixture of compounds with no trace of the product or starting material according to $^1$H-NMR analysis. Most likely these conditions were too acidic, so the reaction was repeated with a lower amount of catalyst. This also gave a mixture of compounds which seemed to be, according to $^1$H spectrum, a mixture of two isomers. A double PMB signal indicates that PMB migration might have happened. However, this is purely speculative and has not been further investigated. After two attempts BF$_3$·Et$_2$O was abandoned and instead TMSOTf was chosen as the new activator. The first attempt with TMSOTf (0.2 eq) gave after purification compound 23 with a yield of 67%. (Appendix A16) After optimizing this reaction was also performed with the glucose and lactose donors. The glucose donor could be coupled with the same ease as galactose to the backbone to obtain compound 24 with a yield of 77%. (Appendix A17) The coupling of the lactose donor 25 proved to be more difficult. The first few attempts gave inseparable mixtures. After
reducing the starting temperature to -10 °C the first NMR spectrum shows one compound, still slightly impure, but only one compound. However, this still has to be purified more and properly confirmed with a full NMR analysis.

2.7 Deprotection of the protected glycosphingosines

The final step before obtaining the glycosphingosines is removing the protecting groups. So far only galactosylsphingosine and glucosylsphingosine have been deprotected. Therefore the deprotection of 3-O-sulphogalactosylsphingosine, which is slightly different, will not be discussed here.

The first step is the removal of the PMB group which is acid labile. It is the reverse reaction of the installment of the PMB group which is thoroughly discussed in subchapter 2.2.4. The reaction is not purified after this step, and therefore, there is not much to say about how well the reaction performs. It is notable that as soon as the reaction starts a deep purple color is observed, this is an indication that the PMB group is falling off. Following this reaction with TLC can be quite troublesome because of the slight impurities that are present in the starting material. The best way to track the progress is to analyze the reaction mixture periodically with an LCMS analysis. However, even then it is still troublesome to clearly see what is going on. In the end, after the second step, 20% TFA/DCM seems enough to remove the PMB group sufficiently. 26

The second step is the removal of all base labile groups. Both the benzoyl and the Fmoc group are base labile. Benzoyl groups will be removed with a simple transesterification reaction. The removal of the Fmoc group is slightly more complex, and a general mechanism can be seen in Figure 32. 26

![Figure 32: Removal of the Fmoc group (mechanism)](image)

Surprisingly it is not the carbonyl that is base labile in this case. Carbamates are more stable than esters and can’t be modified under these conditions. However, the proton attached to the cyclopentadiene ring is base labile. The negative charge forms an aromatic system with the two π-bonds from cyclopentadiene and is stabilized through delocalization. This is called the cyclopentadiene anion and is known to be aromatic. However, the release of CO₂ and the free amine is after this step even more favorable and reaction moves in that direction. 26
3 Conclusion

The desired sphingosine backbone has been synthesized from Fmoc-L-Serine as starting material. The route was based on Yamamoto’s work and the work previously done by P. van Oerle. Fmoc-L-Serine has been transformed into the corresponding Weinreb amide and was protected with TBDMS-Cl. The protected Weinreb amide was transformed in a α-β unsaturated ketone by a Grignard reaction with vinylmagnesiumbromide after which the ketone was stereoselective reduced to the anti-alcohol. This allylic alcohol was protected with a p-methoxybenzyl group after which the 1° alcohol was deprotected with TBAF. Finally, the fatty chain was installed using cross metathesis. The overall yield over 7 steps is 13%.

The route has been further optimized to better suit the need of the route. It is now possible to install the five 13C-atoms in the final step compared to the third last step. This makes the synthesis of the labeled backbone on a large scale more economically favorable.

The second part consisted out of three major steps; synthesis of the individual donors, coupling them to the sphingosine acceptor and the removal of the protecting groups from the coupled compounds. The synthesis of the galactose imidate donor was started with the full benzoylation of D-(+)-galactose. After the anomeric position was selectively deprotected, a trichloroacetimidate group could be installed.

The synthesis of the 3-O-sulfogalactose donor is now in a final stage where an imidate has to be installed on the anomeric position of compound. The first three steps; replacing the anomeric position with thiophenol, deacytelation and the introduction of the benzylidene bridge gave a yield of 80% (over three steps). Introducing the protected sulfate group proved to be somewhat more challenging. So far compound has been synthesized with a yield of 15%, compared to 85% by Desoky et. al. This yield might be improved if the addition of the base happens even slower than a sixth once every hour for six hours. Placing a benzoyl group on the 2-O position gave compound with a yield of 55%. This reaction has been performed twice, with more pyridine the second time. Both reactions gave the same yield. By a lack of time, we didn't optimize this reaction yet. Deprotection of the anomeric position happened without any complication and gave compound with a yield of 90%. Trying to install the imidate on the anomeric position destroyed the starting material. We have some speculations to as why this has happened, but nothing has been investigated yet. Mainly because of a lack of time.

The glucose and lactose imidate donors have been purified with silica gel chromatography and have been tested by NMR. These results showed that the N-H bonds were still in place, which means the imine isn't destroyed, and these donors were used in the following coupling reactions.

The coupling of the galactose imidate donor to the sphingosine tail proved to be more difficult at first. BF₃·Et₂O proved to be either too acidic or acidic enough to give multiple reactions which resulted in respectively a destroyed mixture or a mixture of two inseparable compounds. TMS-OTf proved to be just acidic enough to activate the glycosylation but not enough to mediate all kind of side reaction. It was used to successfully couple galactose and glucose to the sphingosine backbone with yields of respectively 67% and 77%. The coupling of lactose to the sphingosine backbone proved to be more difficult. However, initial NMR results indicate that the compound has been formed. Further purification and analysis are needed. The protected galactosyl- and glucosylsphingosine have been successfully deprotected with a two step synthesis, first acidic conditions to remove the PMB group, followed by basis conditions to remove the benzoysls and Fmoc group.
4 Future prospects

The first step towards a panel of glycosphingolipids is done. The route towards obtaining a $^{13}$C$_5$-labeled sphingosine backbone has been optimized. Now all that remains is to repeat the synthesis, but now we use a fatty tail enriched with five $^{13}$C atoms in the last step. The synthesis of the $^{13}$C$_5$ enriched fatty tail will start from $[^{13}$C] KCN and $[^{13}$C$_2$] acetic acid. This new synthetic route has become the way towards a scaffold that can be used to synthesize a whole range of different glycosphingosines as well as glycosceramides.

The deprotection and purification of the coupled compounds is still in an early phase. They need to be purified by HPLC and thoroughly analyzed with all the available technique.

The synthesis of the 3-O-sulphogalactose donor can be optimized further. Introducing the protected sulfate group will probably go better if a syringe pump is used to add the base over a period. This will most likely ensure a higher selectivity for the 3-O position. Installing the imidate group will probably be achieved quite easily by reducing the basicity of the reaction and therefore eliminating all possible side reactions.
5 Experimental

All reagents and solvents used were obtained from commercial sources except for PMB-imidate and trichlorosulfoimidazolium triflate, they were made from commercially available reagents. Reactions were monitored with TLC using Merck Silica Gel F254 plates. Visualization of the TLC plates was achieved by UV light (λ = 254 nm) in combination with one of the following stains: KMnO4 or Molybdenum oxide spray.

1H and 13C spectra were recorded with a Bruker spectrometer (1H NMR, 400.23 MHz; 13C NMR, 100.65 MHz) in which TMS was used as the internal standard. Chemical shifts are reported in δ (ppm). NMR peak assignments were achieved using a combination of 1H, 13C, COSY and HSQC experiments. Coupling constants (J) are reported in Hz.

Infrared measurements were performed with a Shimadzu FTIR-8400S (neat). Relevant absorbance’s (νmax) are reported in cm⁻¹.

(9H-fluoren-9-yl)methyl (S)-(3-hydroxy-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate (2)

To a solution of DIEA (8.2 mL, 46.9 mmol, 0.9 eq) in DCM (300 mL) Fmoc-L-Serine (17.1 g, 52.2 mmol, 1 eq) was added. The mixture was stirred under argon and cooled down to 0 °C. Next EDC HCl (12 g, 62.6 mmol, 1.2 eq) and HOBt.H2O (9.58 g, 62.6 mmol, 1.2 eq) were added and stirred for 10 minutes. Finally Weinreb salt (6.12 g, 62.6 mmol, 1.2 eq) and DIEA (4.5 mL, 26.1 mmol, 0.5 eq) were added. The mixture was stirred overnight while the temperature rose to room temperature. Afterwards the mixture was washed two times with 2M HCl, two times with sat. NaHCO3 and once with Brine. Aqueous layers were once re-extracted with DCM. The organic layers were combined, dried with MgSO4, filtered and concentrated in vacuo. The resulting crude oil 2 was used in the next step without further purification.

Rf: 0.39 (EtOAc); 1H NMR (400 MHz, CDCl3) δ 7.76 (d, 2H J = 7.5 Hz, Arom), 7.64 – 7.56 (m, 2H, Arom), 7.40 (t, 2H, J = 7.5 Hz, Arom), 7.31 (t, 2H, Arom), 5.92 (d, 1H, J = 8.3 Hz, N-H), 4.87 (s, 1H, H2), 4.40 (d, 2H, J = 7.1 Hz, CH2(Fmoc)), 4.22 (t, 1H, J = 7.0 Hz, CH(Fmoc)), 3.86 (s, 2H, H1), 3.78 (s, 3H, O-CH3), 3.25 (s, 3H, N-CH3), 2.52 (s, 1H, OH); 13C NMR (101 MHz, CDCl3) δ:143.95 (C=O(Fmoc)), 141.46 (C3), 127.84, 127.21, 127.19, 125.25, 120.10 (5x Arom), 67.35 (CH2(Fmoc)), 63.70 (C1), 61.79 (O-CH3), 52.88 (C2), 47.26 (CH(Fmoc)); IR (neat): 3412, 3315 (O-H stretch, N-H stretch), 2941 (C-H saturated), 1714 (C=O stretch), 1448 (C-H bend), 1263 (C=O stretch), 1053 (C-N stretch) cm⁻¹.

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(9H-fluoren-9-yl)methyl (S)-(3,8,8,9,9-pentamethyl-4-oxo-2,7-dioxo-3-aza-8-siladecan-5-yl)carbamate (3)

![Chemical Structure]

To a solution of crude 2 in DMF (500 mL), TBDMS-Cl (10.2 g, 67.6 mmol, 1.3 eq) was added and stirred under argon. The solution was cooled to 0 °C before adding NNm (6.3 mL, 57.2 mmol, 1.1 eq). The mixture was stirred overnight while the temperature rose to room temperature. Afterwards H2O (500 mL) was added. The resulting cloudy solution was extracted twice with Et2O. The organic layers were combined, washed with Brine, dried with MgSO4, filtered and concentrated in vacuo. The resulting crude oil was purified with silica gel chromatography (0-10% EtOAc in pentane) which gave a yellow oil 3 (18.9 g, 39.0 mmol, 75% over two steps).

Rf: 0.95 (EtOAc); 1H NMR (400 MHz, CDCl3) δ 7.77 (d, 2H, J = 7.5 Hz, Arom), 7.62 (t, 2H, J = 8.2 Hz, Arom), 7.40 (t, 2H, J = 7.5 Hz, Arom), 7.32 (t, 2H, J = 7.5 Hz, Arom), 5.72 (d, 1H, J = 8.7 Hz, NH), 4.85 (s, 1H, H2), 4.37 (d, 2H, J = 7.2 Hz, CH2(Fmoc)), 4.25 (t, 1H, J = 7.3 Hz, CHFmoc), 3.96 – 3.83 (m, 2H, H1), 3.77 (s, 3H, O-CH3), 3.25 (s, 3H, N-CH3), 0.90 (s, 9H, t-butyl), 0.06 (s, 6H, 2x Si-CH3); 13C NMR (101 MHz CDCl3); δ143.95 (C=O (fomoc)), 141.46 (C3), 127.82, 127.20, 125.39, 125.34, 120.09, (5x Arom) 67.27 (CH2(Fmoc)), 63.48 (C1), 61.65 (O-CH3), 53.16 (C2), 47.28 (CHFmoc), 32.62 (N-CH3), 25.93 (t-butyl), –5.35 (2x Si-CH3); IR (neat) 3305 (N N stretch), 2953, 2927, 2854 (C-H unsaturated), 1699 (C=O stretch), 1500 (C-H bend Arom), 1450 (C-H bend alkene), 1251, 1026 (C-O stretch) cm⁻¹.

(9H-fluoren-9-yl)methyl (S)-(1-((tert-butyl(dimethyl)silyl)oxy)-3-oxopent-4-en-2-yl)carbamate (4)

THF (24 mL) was dried using activated MS4Å. Product 3 (4.8 g, 10 mmol, 1 eq) was co-evaporated three times with toluene and afterwards dissolved in dry THF under argon. After the solution was cooled down to 0 °C, VinylMgBr (40 mL, 40 mmol, 4 eq) was added and the reaction mixture was left to react for one hour. The reaction mixture was slowly added to cold 2M HCl (200 mL) and quickly extracted with EtOAc (two times). The organic layers were combined, washed with Brine, dried with MgSO4, filtered and concentrated in vacuo. The resulting residue was purified with silica gel chromatography (2.5-10% EtOAc in pentane) which gave a white solid 4 (3 g, 6.7 mmol, 67%).

Rf: 0.75 (15% EtOAc in pentane); 1H NMR (400 MHz, CDCl3) δ 7.77 (d, 2H, J = 7.5 Hz, Arom), 7.62 (t, 2H, J = 6.8 Hz, Arom), 7.41 (t, 2H, J = 7.5 Hz, Arom), 7.32 (t, 2H, J = 7.5, 1.1 Hz, Arom), 6.62 – 6.53 (m, 1H, H4), 6.39 (d, 1H, HSJ, 5.90 – 5.83 (m, 2H, HSJ + NH), 4.72 – 4.67 (m, 1H, H2), 4.39 (d, 2H, J = 7.2 Hz, CH2(Fmoc)), 4.24 (t, 1H, J = 7.2 Hz, CHFmoc), 4.08 – 4.02 (m, 1H, H1a), 3.93 – 3.87 (m, 1H, H1a), 0.86 (s, 9H, t-butyl), 0.02 (d, 6H, J = 3.7 Hz, 2x Me-Si); 13C NMR (101 MHz CDCl3); 155.96 (C3), 143.93 (C=O (fomoc)), 133.14 (C4), 129.9 (C5), 127.85, 127.21, 125.30, 125.26, 126.02 (5x Arom), 67.26 (CH2(Fmoc)), 63.47 (C1), 60.05 (C2), 47.31 (CHFmoc), 25.86 (t-butyl), –5.44 (2x Si-CH3); IR (neat) 2953, 2927, 2854 (C-H stretch saturated) 1699 (C=O stretch), 1500 (C-C stretch Arom), 1450 (C-H bend alkene), 1251, 1026 (C-O stretch) cm⁻¹.
(9H-fluoren-9-yl)methyl ((2S,3R)-1-((tert-butyldimethylsilyl)oxy)-3-hydroxypent-4-en-2-yl)carbamate (5)

To a solution of 4 (3.0 g, 6.7 mmol, 1 eq) in EtOH (500 mL) LiAlH(OtBu)₃ (3.7 g, 14.7 mmol, 2.2 eq) was added at -78 °C and stirred under argon. After 5 hours the mixture was quenched with 176 mL 0.1 M HCl and quickly extracted with EtOAc (two times). The organic layers were washed with 0.1 M HCl and Brine, dried with MgSO₄, filtered and concentrated in vacuo. The crude oil was purified with silica gel chromatography (2.5-10% EtOAc in pentane) which gave two white solids: 5 (2.2 g, 4.9 mmol, 73%) and starting material (0.7 g, 1.6 mmol, 23%).

Rf: 0.52 (15% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, 2H, J = 7.5 Hz, Arom), 7.63 – 7.56 (m, 2H, Arom), 7.40 (t, 2H, J = 7.5 Hz, Arom), 7.34 – 7.28 (m, 2H, Arom), 5.99 – 5.89 (m, 1H, H₄), 5.56 (d, 1H, J = 8.6 Hz, NH), 5.41 (d, 1H, J = 17.1 Hz, H₅a), 5.27 (d, 1H, J = 10.6 Hz, H₅b), 4.39 (d, 2H, J = 7.2, 2.6 Hz, CH₂(Fmoc)), 4.31 (s, 1H, H3), 4.24 (t, 1H, J = 7.0 Hz, CH₆(Fmoc)), 3.97 (d, 1H, J = 10.4, 2.8 Hz, H₁a), 3.78 (d, 1H, J = 10.3, 3.2 Hz, H₁b), 3.37 (s, 1H, H2) 0.91 (s, 9H, t-Butyl), 0.07 (s, 6H, 2x Si-CH₃); ¹³C NMR (101 MHz CDCl₃); δ 143.98 (C=O Fmoc), 137.34 (C4), 127.34 (C5), 116.36 (C5), 74.88 (C3), 66.95 (CH₂(Fmoc)), 62.35 (C1), 55.19 (C2), 47.34 (CH₆(Fmoc)), 25.94 (t-butyl), -5.50 (2x Si-CH₃); IR (neat): 3439 (N-H stretch), 2927, 2854 (C-H stretch saturated), 1705 (C=O stretch Fmoc), 1251 (C-N stretch), 1080 cm⁻¹ (C-O stretch ether, stretch ester) cm⁻¹.
Compound 5 (0.11 g, 0.24 mmol, 1 eq), PMB-NPTFA (0.11 g, 0.36 mmol, 1.5 eq) and stirring bar were co-evaporated thrice with toluene under argon. Activated powdered MS4Å were added together with dry toluene (1 mL). 10-Camphorsulphonic acid (0.007 g, 0.024 mmol, 0.1 eq) was added and the mixture was left to react overnight while the temperature rose to room temperature. The mixture was filtered over Celite, washed with NaHCO₃ and Brine, dried over MgSO₄, and concentrated in vacuo. The product was purified with silica gel chromatography (2.5-10 % EtOAc in pentane) which gave slightly yellow solid 6 (0.08 g, 0.13 mmol, 58%)

Rf: 0.5 (10% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, 2H, J = 7.5 Hz, Arom), 7.57 (d, 2H, J = 7.4 Hz, Arom), 7.39 (t, 2H, J = 7.5 Hz, Arom), 7.29 (t, 2H, J = 7.5 Hz, Arom), 7.23 (d, 2H, J = 7.5 Hz, Arom), 6.89 – 6.83 (m, 2H, Arom), 5.89 – 5.77 (m, 1H, H4), 5.37 – 5.27 (m, 2H, H5a + NH), 5.06 (d, 1H, J = 9.7 Hz, H5b), 4.54 (d, 1H, J = 11.3 Hz, H3), 4.39 – 4.19 (m, 5H, CH₂(Fmoc) + CH₂(Fmoc) + CH₂(PMB)), 3.96 – 3.87 (m, 2H, H1a + H2), 3.78 (d, 3H, J = 2.9 Hz, CH₃(PMB)), 3.65 (dd, 1H, J = 10.0, 4.1 Hz, H1b), 0.90 (d, 9H, J = 2.9 Hz, t-Butyl), 0.06 (d, 6H, J = 4.0 Hz, 2x Si-CH₃); ¹³C NMR (101 MHz, CDCl₃) δ 156.32 (C=O_Fmoc), 137.74 (C4), 127.83, 127.18, 125.25, 125.20, 120.12 (5x Arom), 116.33 (C5), 74.81 (C3), 67.06 (CH₂(Fmoc)), 63.50 (C1), 54.52 (C2), 47.32 (CH₂(Fmoc)), 25.92 (t-butyli), -5.50 (2x Si-CH₃); IR (neat): 3444 and 3317 (N-H stretch), 3070 (C-H unsaturated), 2950 and 2854 (C-H saturated), 1712 (C=O Fmoc), 1242 (C-N stretch) cm⁻¹.
(9H-fluoren-9-yl)methyl (2S,3R)-1-hydroxy-3-((4-methoxybenzyl)oxy)pent-4-en-2-yl)carbamate (7)

To a solution of 6 (0.25 g, 0.43 mmol, 1 eq) in 2 mL THF, acetic acid (0.1 mL, 1.7 mmol, 4 eq) was added under argon. After stirring TBAF 1M in THF (0.9 mL, 0.86, 2 eq) was added and the mixture was left to react overnight at room temperature. The solution was diluted with EtOAc, washed with NaHCO₃, 1M HCl, and Brine, dried with MgSO₄ and concentrated in vacuo. The crude mixture was purified with silica gel chromatography (10-40 % EtOAc in pentane) which yielded a white solid 7 (0.19 g, 0.4 mmol, 94 %).

Rf: 0.05 (20% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.76 (d, 2H, J = 7.6 Hz, Arom), 7.63 – 7.55 (m, 2H, Arom), 7.44 – 7.37 (m, 2H, Arom), 7.35 – 7.29 (m, 2H, Arom), 7.22 (d, 2H, J = 8.3 Hz, Arom), 6.86 (d, 2H, J = 7.9 Hz, Arom), 5.88 – 5.76 (m, 1H, H4), 5.52 (d, 1H, J = 8.5 Hz, H5s), 5.44 – 5.35 (m, 2H, H5a + NH), 4.59 (d, 1H, J = 11.3 Hz, CHFmoc), 4.41 – 4.32 (m, 2H, CH₂(PMB)), 4.27 – 4.17 (m, 2H, CH₂(Fmoc)), 4.13 – 4.07 (m, 1H, H3), 4.01 (d, 1H, J = 11.6 Hz, H2), 3.76 (s, 3H, CH₃(PMB)), 3.71 – 3.59 (m, 2H, H1₄ + H1₅), IR (neat): 3487 and 3340 (N-H and NH) 3050 (C-H saturated), 2900-2870 (C-H unsaturated), 1674 (C=O), 1250 (C-N) cm⁻¹.

(9H-fluoren-9-yl)methyl (2S,3R,E)-1-hydroxy-3-((4-methoxybenzyl)oxy)octadec-4-en-2-yl)carbamate (8)

To a solution of 7 (0.09 g, 0.19 mmol, 1 eq) in 2 mL DCM, 1-pentadecene (0.16 mL, 0.38 mmol, 2 eq) and acetic acid (0.011 mL, 0.19 mmol, 1 eq) were added and stirred under argon. Next Grubbs II (0.016 mg, 0.019 mmol, 0.1 eq) was added and the mixture was heated to 40 °C. After two days the solution was concentrated in vacuo and the crude residue was purified with silica gel chromatography (0-15 % EtAOc in pentane) which yielded a white solid 8 (0.080 g, 0.17 mmol, 65%).

Rf: 0.31 (30% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.76 (d, 2H, J = 7.7, 2.1 Hz, Arom), 7.63 – 7.55 (m, 2H, Arom), 7.43 – 7.36 (m, 2H, Arom), 7.35 – 7.28 (m, 2H, Arom), 7.24 – 7.17 (m, 2H, Arom), 6.87 – 6.81 (m, 2H, Arom), 5.83 – 5.74 (m, 5H), 5.64 (m, 1H, J = 8.3 Hz, N-H), 5.48 – 5.39 (m, 1H, H4), 4.56 (d, 1H, J = 11.4 Hz, CHFmoc), 4.38 – 4.29 (m, 2H, CH₂(PMB)), 4.25 – 4.15 (m, 2H, CH₂(Fmoc)), 4.07 – 3.97 (m, 2H, H3 + H1₄), 3.74 (s, 3H, CH₃(PMB)), 3.71 – 3.60 (m, 2H, H2 + H1₅), 2.14 – 2.02 (m, 2H, H6), 1.26 (s, 26H, H7-H17), 0.88 (t, 3H, J = 6.7 Hz, H18); ¹³C NMR (101 MHz, CDCl₃) δ 144.03 (C=O(Fmoc)), 137.16 (C4), 129.88, 127.80, 126.52, 125.23, 120.08, 114.03 (6x Arom), 81.61 (CH₂(Fmoc)), 70.44 (CH₃(Fmoc)), 66.95 (CH₃(PMB)), 62.96 (C3), 62.19 (C1), 55.33 (CH₃(PMB)), 47.35 (CH₃(Fmoc)), 32.46 (C6), 32.05, 29.83, 29.81, 29.79, 29.76, 29.70, 29.58, 29.49, 29.43, 29.32, 29.22, 28.72 (C7-C17), 14.26 (C18); IR: 3464 and 3325 (N-H and OH), 2924 and 2854 (C-H saturated), 1689 (C=O), 1249 (C-N), 1233 (C-O) cm⁻¹.
(2S,3R,4S,5S,6R)-6-((benzoyloxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetrabenzoate (10)

To a solution of 9 (1.80 g, 10 mmol, 1 eq) in pyridine (100 mL), benzoyl chloride (8.35 mL, 60 mmol, 6 eq) was added and stirred at 0 °C under argon. The reaction mixture was left to react overnight. It was quenched with MeOH (2 mL) and concentrated in vacuo. Then it was dissolved in EtOAc, washed with 1M HCl, sat. NaHCO₃ and Brine, and dried with MgSO₄. After evaporation the crude mixture was purified by recrystallization in DCM/EtOH which yielded a white solid 10 (7.00 g, 10 mmol, quant.)

Rₓ: 0.7 (40% EtOAc in pentane); ¹H NMR (400 MHz CDCl₃): δ 8.14–7.24 (25 H, Arom), 6.20 (d, 1 H, J = 2.8 Hz, H4), 6.15 (d, 1 H, J = 3.2, H2) 4.85 (t, 1 H, J = 6.4 Hz, H5) 4.64 (dd, 1 H, J =11.2, 6.4 Hz, H6a) 4.44 (dd, 1 H, J = 11.2, 7.2 Hz, H6b) 3.95 (s, 1H, OH); ¹³C NMR (101 MHz CDCl₃) δ 166.00, 165.80, 165.65, 165.58, 164.63 (5x O=O benzoyl), 134.02, 133.84, 133.59, 133.51, 133.35, 130.07, 130.04, 129.85, 129.38, 129.06, 128.91, 128.88, 128.84, 129.77, 128.82, 128.51. 128.46 (18x Arom) 90.76 (C1) 69.53 (C5), 68.56 (C3), 68.54 (C4), 67.77 (C2), 61.92 (C6); IR (neat): 1722 (C=O stretch) 1259 (C–O stretch) 1091, 1066, 1026 (C–O stretch) cm⁻¹.

(2R,3S,4S,5R,6R)-2-((benzoyloxy)methyl)-6-hydroxytetrahydro-2H-pyran-3,4,5-triyl tribenzoate (11)

To a solution of 10 (6.8 g, 9.7 mmol, 1 eq) in DMF (100 mL), hydrazine acetate (1.78 g, 19.4 mmol, 2 eq) was added and stirred under argon. The reaction mixture was left to react overnight. After adding H₂O (200 mL), the solution was extracted wit Et₂O, washed with Brine and dried with MgSO₄. The organic layers were combined and concentrated in vacuo. The crude oil was purified with silica gel chromatography (10-40 % EtOAc in pentane) which yielded a colourless oil 11 (5.2 g, 8.7 mmol, 89%).

Rₓ: 0.62 (40% EtOAc in pentane) (α-β mixture, ratio not determined.); β ¹H NMR (400 MHz CDCl₃) δ 8.12 – 8.07 (m, 3H, Arom), 8.03 – 7.94 (m, 6H, Arom), 7.82 – 7.76 (m, 3H, Arom), 7.64 – 7.56 (m, 2H, Arom), 7.55 – 7.30 (m, 13H, Arom), 7.29 – 7.19 (m, 5H, Arom), 7.19 – 7.12 (m, 2H, Arom), 6.12 – 6.05 (m, 2H, H1 & H4), 5.85 (d, 1H, J = 2.6 Hz, H2), 5.74 – 5.71 (m, 1H, H3), 4.86 (t, 1H, J = 6.6 Hz, H5), 4.60 (dd, 1H, J = 11.3, 6.4 Hz, H6a), 4.37 (dd, 1H, J = 11.3, 6.7 Hz, H6b), 3.95 (s, 1H, OH); ¹³C NMR (101 MHz CDCl₃) δ 166.96, 166.30, 166.21, 165.73, 165.64 (5x C=O benzoyl), 133.70, 133.64, 133.51, 133.43, 133.34, 133.27, 130.08, 130.03, 129.99, 129.94, 129.89, 129.85, 129.80, 129.13, 128.74, 128.54, 128.50, 128.42, 128.35, 128.32 (20x Arom), 91.19 (C2), 69.69 (C3), 69.42 (C4), 68.17 (C5), 66.85 (C6a), 62.50 (C6b); IR (neat) 3070 (CH stretch Arom), 1724 (C=O stretch), 1267 (C-O stretch) cm⁻¹.
(2R,3S,4S,5R,6S)-2-((benzoyloxy)methyl)-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (12)

To a solution of 11 (5.2 g, 8.7 mmol, 1 eq) in DCM (40 mL), trichloroacetonitrile (3.48 mL, 34.8 mmol, 4 eq) and K$_2$CO$_3$ (3.6 g, 26.1 mmol, 3 eq) were added and stirred under argon. The reaction mixture was left to stir overnight. After TLC showed full conversion of the starting material the reaction mixture was filtered over Celite, concentrated in vacuo and purified silica gel chromatography (10-50% EtOAc in pentane, 2% Et$_3$N). This yielded 12 as a white foam. (4.0 g, 5.4 mmol, 60%) 

R$_f$: 0.74 (40% EtOAc, 2% Et$_3$N in pentane); (α-β mixture, ratio not determined, NMR shows mainly β); 

$^1$H NMR (400 MHz CDCl$_3$): 8.64 (s, 1 H, NH), 8.13–7.31 (m, 20 H, arom), 6.91 (d, 1 H, $J = 3.6$ Hz, H$_1$), 6.16 (m, 1 H, H$_4$), 6.09 (dd, 1 H, J = 10.8, 3.2 Hz, H$_3$), 5.85 (dd, 1 H, J = 10.4, 3.6 Hz, H$_2$), 4.87 (t, 1 H, J = 6.4 Hz, H$_5$), 4.62 (dd, 1 H, J = 11.2, 6.8 Hz, H$_6_a$), 4.42 (dd, 1 H, J = 11.6, 6.0 Hz, H$_6_b$)$_6$; $^{13}$C NMR (101 MHz CDCl$_3$) 166.00, 165.71, 165.60, 165.53 (4x C=O benzoyl), 160.70 (C=NH imidate), 133.97–128.43 (Arom), 93.80 (C1), 69.77 (C5), 68.57 (C4), 68.57 (C3), 67.90 (C2), 62.26 (C6); IR (neat) 3344 (N–H stretch), 1720 (C=O stretch), 1259 (C–O stretch) cm$^{-1}$.

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((phenylthio)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (14)

To a solution of 13 (9.8 g, 25 mmol, 1 eq) in 195 mL DCM, thiophenol (3.9 mL, 37.5 mmol, 1.5 eq) was added and stirred under argon. After cooling the reaction mixture to 0°C, BF$_3$.Et$_2$O (4.82 mL, 37.5 mmol, 1 eq) was added dropwise. The ice bath was removed and the solution was left to react for 3 hours. After 3 hours TLC (50% EtOAc in pentane) showed full conversion of the starting material. The mixture was washed with sat. NaHCO$_3$ and Brine, dried with MgSO$_4$ and the organic layer was concentrated in vacuo. The crude oil was purified with silica gel chromatography (25-30% EtOAc in pentane) to obtain a white solid 14 (11 g, 25 mmol, quant.) 

R$_f$: 0.75 (50% EtOAc in pentane); $^1$H NMR (400 MHz CDCl$_3$) $\delta$ 7.54 – 7.49 (m, 2H, Arom), 7.34 – 7.30 (m, 3H, Arom), 5.42 (d, 1H, J = 3.3 Hz, H$_4$), 5.24 (t, 1H, J = 10.0 Hz, H$_2$), 5.06 (dd, 1H, J = 10.0, 3.3 Hz, H$_3$), 4.74 (d, 1H, J = 10.0 Hz, H$_1$), 4.22 – 4.09 (m, 2H, H$_6_a$b), 3.96 (t, 1H, J = 6.9 Hz, H$_5$), 2.12 (s, 3H, CH$_3$ Acetyl), 2.10 (s, 3H, CH$_3$ Acetyl), 2.04 (s, 3H, CH$_3$ Acetyl), 1.97 (s, 3H, CH$_3$ Acetyl); $^{13}$C NMR (101 MHz CDCl$_3$) $\delta$ 170.35, 170.18, 170.02, 169.42, (4x Acetyl) 132.47, 128.89, 128.14, (5x Arom) 86.52 (C1), 74.39 (C5), 71.97 (C3), 67.34, 67.22, (C2 and C4) 61.65 (C6), 20.85, 20.67, 20.64, 20.59 (4x C Acetyl); IR (neat) 3064 (C–H stretch Arom), 2920, 2856 (C–H stretch saturated), 1716 (C=O stretch), 1039, 1008 (C–O stretch ester) cm$^{-1}$. 

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(2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-((phenylthio)oxy)tetrahydro-2H-pyran-3,4,5-triol (15)

To a solution of 14 (11 g, 25 mmol, 1 eq) in MeOH, a few drops of NaOMe 30% in MeOH were added and reaction mixture was left stirring under argon overnight. TLC (1:1 EtOAc:pentane) showed full conversion and the mixture was quenched with amberlite (H⁺ donor), filtered and concentrated in vacuo. This yielded the white solid 15 (6.5 g, 23.8 mmol, 95%).

Rf: 0 (50% EtOAc in pentane); ¹H NMR (400 MHz, MeOD) δ 7.58 (dd, J = 8.3, 1.1 Hz, 2H, Arom), 7.36 – 7.23 (m, 3H, Arom), 4.62 (d, 1H, J = 9.7 Hz, H1), 3.94 (d, 1H, J = 2.9 Hz, H4), 3.77 (qd, 2H, J = 11.5, 6.1 Hz, H6), 3.68 – 3.58 (m, 2H, H2 + H5), 3.54 (dd, 1H, J = 9.2, 3.3 Hz, H3); ¹³C NMR (101 MHz, MeOD) δ 136.85, 132.88, 130.72, 128.87 (5x Arom), 91.09 (C1), 81.40 (C2), 71.11 (C3), 71.82, 71.20 (C4 + C5), 63.39 (C6); IR (neat) 3309, 2914, 2866 (C-H stretch saturated), 1463, 1438 (C=C stretch Arom) cm⁻¹.

(2S,4aR,6S,7R,8R,8aR)-2-phenyl-6-((phenylthio)oxy)hexahydropyrano[3,2-d][1,3]dioxine-7,8-diol (16)

To a solution of 15 (6.3 g, 23.2 mmol, 1 eq) in ACN/DMF, PhCH(OMe)₂ (5.2 mL, 34.7 mmol, 1.5 eq) and a spatula pTsOH were added. The flask was connected to a rotavap and placed under reduced pressure (350 mbar). The reaction was heated to 50°C and stirred for 2.5 hours. More PhCH(OMe)₂ (0.9 mL, 5.8 mmol, 0.25 eq) was added and the reaction was left to stir under the same conditions for another hour. The ACN was evaporated in vacuo after which the reaction mixture was quenched with a few drops of Et₃N. The resulting mixture was diluted with EtOAc, washed with brine and the co-evaporated with toluene in vacuo. Purification with silica gel chromatography (100:1 -> 50:1 -> 20:1 DCM:MeOH) yielded a white solid 16 (6.8 g, 18.9 mmol, 84%).

Rf: 0.4 (100% EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 7.67 (d, J = 6.6 Hz, 2H, Arom), 7.41 – 7.34 (m, 5H, Arom), 7.32 – 7.24 (m, 3H, Arom), 5.48 (s, 1H, CH-Ph), 4.47 (d, 1H, J = 9.0 Hz, H1), 4.35 (dd, H1, J = 12.4, 1.6 Hz, H5), 4.15 (d, J = 2.0 Hz, 1H), 3.99 (dd, J = 12.5, 1.6 Hz, 1H), 3.66 (d, J = 8.0 Hz, 2H), 3.49 (s, 1H), 2.79 (s, 2H); ¹³C NMR (126 MHz, CDCl₃) δ 133.92, 129.52, 129.11, 128.39, 126.65, 101.57, 75.44, 73.93, 73.93, 70.21, 69.43, 68.95; IR (neat) 3398, 3313 (O-H stretch), 2914, 2870 (C-H stretch saturated), 1006 (C=C stretch Arom) cm⁻¹.
(2S,4aR,6S,7R,8R,8aS)-7-hydroxy-2-phenyl-6-((phenylthio)oxy)hexahydropyrano[3,2-d][1,3]dioxin-8-yl (2,2,2-trichloroethyl) sulfate (17)

To a solution of 16 (5.8 g, 16.3 mmol, 1 eq) in dry DCM (50 mL, 0.3M), TCES-imidazolium triflate (14.9 g, 32.5 mmol, 2 eq) was added at 0°C and stirred under argon. Next a solution of DMI (3.9 g, 40.6 mmol, 2.5 eq) in dry DCM (25 mL) was added portion wise over a period of 6 hours. The ice bath was removed and the reaction mixture was left to react over the weekend. The reaction mixture was washed with Brine, dried with MgSO₄ and concentrated in vacuo. The crude brown oil was purified with silica gel chromatography (5-20% EtOAc in pentane) to obtain compound 17 (1.35 g, 2.4 mmol, 15%)

Rf: 0.5 (20% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.70 – 7.65 (m, 2H, Arom), 7.57 – 7.51 (m, 1H, Arom), 7.39 – 7.32 (m, 7H, Arom), 7.29 (dd, 2H, J = 7.3, 1.1 Hz, Arom), 5.54 (s, 1H, H7), 4.85 (d, 1H, J = 10.8 Hz, H8a), 4.71 (dd, 1H, J = 9.6, 3.5 Hz, H3), 4.66 (d, 1H, J = 10.8 Hz, H8b), 4.59 (dd, 1H, J = 4.4, 1.7 Hz, H4), 4.57 – 4.53 (m, 1H, H1), 4.40 (dd, 1H, J = 12.5, 1.6 Hz, H6a), 4.00 – 3.92 (m, 2H, H2), 3.62 – 3.59 (m, 1H, H5); ¹³C NMR (101 MHz, CDCl₃) δ 137.19, 134.15, 132.91, 129.53, 129.43, 129.39, 129.32, 128.36, 126.56 (10x Ph), 101.23 (C7), 88.92 (C1), 80.06, 79.98 (C8), 73.55 (C4), 69.72 (C5), 69.09 (C6a+b), 65.45 (C2); IR (neat) 3340, 3298 (O-H stretch), 2916, 2862 (C-H stretch saturated), 1408 (S=O stretch), 1006 (C=C stretch Arom) cm⁻¹.

To a solution of 17 (1.35 g, 2.4 mmol, 1 eq) in 30% pyridine/DCM (100 mL), benzoyl chloride (0.67 mL, 4.8 mmol, 2 eq) and a catalytic amount of DMAP were added and stirred under argon at 0°C. The reaction mixture was left stirring overnight while the temperature rose to room temperature. The reaction was quenched with H₂O (1 mL) and concentrated in vacuo after which it was co-evaporated with toluene to remove any residual pyridine. The resulting crude mixture was purified with silica gel chromatography (5-20% EtOAc in pentane) to obtain a white solid 18 (0.9 g, 1.3 mmol, 55%).

Rf: 0.3 (20% EtOAc in Pentane + a few drops of Et₃N); ¹H NMR (400 MHz, CDCl₃) δ 8.07 (d, 2H, J = 8.5 Hz, Arom), 7.59 (t, 2H, J = 1.6 Hz, Arom), 7.48 (t, 2H, J = 7.7 Hz, Arom), 7.39 (s, 6H, Arom), 7.32 – 7.25 (m, 3H, Arom), 5.63 – 5.56 (m, 2H, H2 & H7), 5.01 (dd, 1H, J = 9.8, 3.5 Hz, H3), 4.87 (d, 1H, J = 9.7 Hz, H1), 4.69 (d, 1H, J = 3.5 Hz, H4), 4.53 – 4.41 (m, 3H, H6a & H8), 4.12 – 4.06 (m, 1H, H6b), 3.68 (s, 1H, H5); ¹³C NMR (101 MHz, CDCl₃) δ 136.90, 134.52, 133.78, 130.13, 129.71, 129.03, 128.77, 128.48, 126.72 (9x Arom), 101.55 (C7), 85.13 (C3), 80.08 (C8), 69.55 (C5), 69.13 (C6a), 66.77 (C6b); IR (neat) 3396, 3313 (O-H stretch), 1006 (C=C stretch) cm⁻¹.
**To a solution of 18 (0.35 g, 0.5 mmol, 1 eq) in DCM (7 mL, 0.1M), NIS (0.13 g, 0.6 mmol, 1.1 eq) and TFA (0.05 mL, 0.6 mmol, 1.1 eq) were added and stirred under argon at 0 °C. The reaction mixture was left to stir for a hour at room temperature and then checked with TLC which showed full conversion into a lower running spot. The reaction mixture was then cooled back to 0 °C and piperidine (0.15 mL, 0.1 mmol, 3 eq) was added. The reaction mixture was left to stir for another hour at room temperature and then transferred into a separation funnel with DCM. It was washed with sat. Na₂S₂O₃, 1M HCl, sat. NaHCO₃ and Brine. The organic layers were combined and dried with MgSO₄, filtered off and concentrated in vacuo. The resulting crude mixture was purified with silica gel chromatography (30% EtOAc in pentane) to obtain a slightly yellow oil 19 (0.28 g, 0.5 mmol, 90%).**

**R:** 0.4 (30% EtOAc in Pentane); **¹H NMR (400 MHz, CDCl₃) δ 8.08 (d, 2H, J = 8.3 Hz, Arom), 7.61 – 7.10 (m, 14H, Arom), 5.72 – 5.66 (m, 1H, H1), 5.60 – 5.51 (m, 2H, H2 & H7), 5.42 (dd, 1H, J = 10.8, 3.1 Hz, H3), 4.66 (d, 1H, J = 3.0 Hz, H4), 4.58 (s, 2H, H8), 4.25 (d, 1H, J = 12.6 Hz, H6a), 4.09 – 3.97 (m, 2H, H6b & H5); **¹³C NMR (101 MHz, CDCl₃) δ 165.91, 136.92, 133.82, 130.15, 130.05, 129.73, 129.57, 129.13, 129.05, 128.78, 128.55, 128.49, 128.32, 126.72, 126.33, 126.26, 125.39, 101.12, 95.80, 92.52, 91.13, 81.49, 80.04, 79.93, 79.34, 73.87, 73.24, 70.89, 69.15, 68.53, 61.94; IR (neat) 3404, 3251 (O–H stretch), 2914, 2866 (C–H stretch saturated), 1006 (C=C stretch) cm⁻¹.

**Compound 7 (0.10 g, 0.16 mmol, 1 eq) and compound 12 (0.24 g, 0.32 mmol, 2 eq) were co-vapped three times with toluene before being dissolved in 1 mL dry DCM. After dissolving grounded MS were added and the reaction mixture was left to stir for 30 minutes while cooling it down to -5 °C with an ice bath. TMSOTf (0.006 mL, 0.0032 mmol, 0.02 eq) was added after which the ice bath was removed and the reaction mixture was left to react for 4 hours. After TLC (20% EtOAc in pentane) showed full conversion of 7 the reaction mixture was quenched with a drop of Et₃N before being filtered of Celite. The reaction mixture was concentrated in vacuo and purified with silica gel chromatography (10-20% EtOAc in pentane) and SEC (1:1 DCM:Methanol). This yielded a white solid 13 (0.13 g, 0.01 mmol, 67%).**

**R:** 0.3 (20% EtOAc in pentane); **¹H NMR (400 MHz, CDCl₃) δ 8.09 (d, 2H, J = 7.2 Hz), 8.06 – 7.99 (m, 3H), 7.90 (d, 2H, J = 7.4 Hz), 7.82 – 7.73 (m, 5H), 7.64 – 7.16 (m, 26H), 6.84 (d, 2H, J = 8.7 Hz), 6.02 (d, 2H, J = 10.4, 3.4 Hz), 5.49 – 5.40 (m, 1H), 5.28 (dd, 1H, J = 15.4, 7.8 Hz), 4.91 (d, 1H, J = 8.8 Hz), 4.75 (d, 1H, J = 7.8 Hz), 4.66 (dd, 1H, J = 11.3, 6.4 Hz), 4.50 – 4.43 (m, 2H),
4.40 – 4.26 (m, 4H), 4.16 – 4.04 (m, 2H), 3.89 – 3.75 (m, 2H), 3.73 (d, J = 5.9 Hz), 1.91 (d, 2H, J = 6.9 Hz), 1.33 – 1.15 (m, 27H), 0.88 (t, 4H, J = 6.8 Hz); 13C NMR (101 MHz, CDCl3) δ 166.11, 165.61, 165.44, 159.19, 155.84, 141.34, 143.94, 137.28, 133.68, 133.40, 130.47, 130.09, 129.86, 129.68, 129.43, 129.39, 129.12, 128.86, 128.74, 128.58, 128.39, 127.77, 127.13, 125.20, 125.13, 120.08, 113.91, 113.83, 102.02, 79.06, 77.48, 77.16, 76.84, 71.62, 71.47, 70.25, 70.18, 68.58, 68.18, 66.50, 62.14, 55.27, 53.95, 47.34, 32.33, 32.02, 29.81, 29.76, 29.56, 29.47, 29.30, 29.25, 22.80, 14.25; IR (neat) 3406 (N-H stretch), 2912, 2868 (C-H saturated), 1732 (C=O stretch), 1066, 1039, 1006, 995 (C=C stretch) cm⁻¹.


Experimental: see compound 23.

Rf: 0.4 (20% EtOAc in pentane); 1H NMR (400 MHz, Chloroform-d) δ 8.05 – 7.72 (m, 14H), 7.56 – 7.16 (m, 29H), 6.81 (d, J = 8.6 Hz, 2H), 5.95 (t, J = 9.6 Hz, 1H), 5.71 (t, J = 9.7 Hz, 1H), 5.57 – 5.42 (m, 2H), 5.28 (dd, J = 15.2, 8.4 Hz, 1H), 4.89 (d, J = 9.0 Hz, 1H), 4.79 (d, J = 7.8 Hz, 1H), 4.66 (dd, J = 12.1, 3.0 Hz, 1H), 4.54 – 4.41 (m, 2H), 4.37 – 4.23 (m, 3H), 4.11 (dt, J = 31.1, 6.5 Hz, 3H), 3.83 (t, J = 19.4 Hz, 2H), 3.74 (d, J = 4.1 Hz, 4H), 3.68 (dd, J = 10.0, 3.4 Hz, 1H), 1.91 (d, J = 6.9 Hz, 2H), 1.22 (d, J = 16.9 Hz, 27H), 0.88 (t, J = 6.8 Hz, 4H); 13C NMR (101 MHz, CDCl3) δ 166.24, 165.86, 165.37, 165.34, 159.19, 155.88, 144.14, 143.99, 141.41, 141.35, 137.35, 133.60, 133.45, 133.39, 133.24, 130.46, 129.95, 129.93, 129.85, 129.60, 129.49, 129.10, 128.92, 128.87, 128.56, 128.49, 128.44, 127.81, 127.78, 127.15, 125.29, 125.22, 120.11, 120.08, 113.83, 101.68, 79.08, 72.82, 72.36, 72.33, 70.32, 69.81, 68.55, 66.67, 63.28, 53.86, 47.33, 32.35, 32.05, 29.84, 29.82, 29.79, 29.58, 29.50, 29.33, 29.28, 22.83, 14.27. IR (neat) 3406 (N-H stretch), 2920, 2860 (C-H stretch saturated), 1728, 1716 (C=O stretch), 1083, 1066, 995 (C=C stretch) cm⁻¹.

(2R,3R,4S,5R,6R)-2-(((2S,3R,E)-2-amino-3-hydroxyoctadec-4-en-1-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (26)

To a cold (0 °C) solution of compound 21 (0.27 g, 0.22 mmol, 1 eq) in 1 mL DCM was added 2.5 mL 20% TFA in DCM while stirring under argon atmosphere. The reaction mixture was stirred for 12 hours and then quenched with solid NaHCO₃, filtered and concentrated in vacuo. This crude reaction mixture was used in the next step without any further purification. The crude mixture was dissolved in 3 mL MeOH and added to 1,7 mL 1M NaOH in MeOH solution. The reaction mixture was stirred for 12 hours, quenched with a few drops of acetic acid and concentrated in vacuo. The crude mixture was purified with silica gel chromatography (10% MeOH/DCM -> 30% MeOH/DCM -> 3% H₂O/27 % MeOH/70% DCM) to obtain slightly impure compound 26 (0.10 g, 0.21 mmol, 98%)*
Rt: 0.5 (3% H₂O/27 % MeOH/70% DCM); LCMS ES-API for compound 26 (M+H⁺); calculated 462,34; found 462.4.

*Yield is most likely a little bit lower, NMR analysis did not give a clear spectrum, however, MS analysis shows mostly the product.

\[\text{(2R,3R,4S,5S,6R)-2-(((2S,3R,E)-2-amino-3-hydroxyoctadec-4-en-1-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (27)}\]

Experimental: see compound 26.
Yield: enough for analysis (5-10 mg).
LCMS ES-API for compound 27 (M+H⁺); calculated 462,34; found 462.4.
6 Literature list


(33) de Geus, M. A. R. Synthetic methodoly (methathese), 2014.


Appendix

I: $^1$H and $^{13}$C spectra

A1: compound 3
A2: compound 4
A3: compound 5

TBDMISO

HNFmoc

OH

7.5  7.0  6.5  6.0  5.5  5.0  4.5  4.0  3.5  3.0  2.5  2.0  1.5  1.0  0.5  0.0

-13000  -12000  -11000  -10000  -9000  -8000  -7000  -6000  -5000  -4000  -3000  -2000  -1000  0  1000  2000  3000  4000  5000  6000

TBDMISO

HNFmoc

OH

60  120  180  240  300  360  420  480  540  600  660  720  780  840  900  960  1020  1080  1140  1200

-6000  -5000  -4000  -3000  -2000  -1000  0  1000  2000  3000  4000  5000  6000
A5: compound 7
A6: compound 8
A7: compound 10
A8: compound 11
A9: compound 12
A10: compound 14
A11: compound 15

[Chemical structure image]

[Graphical data]
A13: compound 17
A14: compound 18
A16: compound 23

[Chemical structure image]

66
A17: compound 24