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The synthesis of chemical tools for studying sphingolipid metabolism

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The Synthesis of Chemical Tools for Studying Sphingolipid Metabolism

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden
op gezag van Rector Magnificus Prof. mr. C. J. J. M. Stolker,
volgens besluit van het College voor Promoties
te verdediging op donderdag 18 januari 2018
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Tabel of contents

Chapter 1

| | |
|---|---|
| Chemical Tools to Study Sphingolipid Metabolism | 5 |
|---|---|

Chapter 2

| | |
|---|----|
| Synthesis of a Panel of Carbon-13-Labeled (Glyco)Sphingolipids | 19 |
|---|----|

Chapter 3

| | |
|--|----|
| <i>N</i> -Fmoc-Protected Sphingosine is a Suitable Starting Material in the Synthesis of Glycosylsphingosines | 51 |
|--|----|

Chapter 4

| | |
|---|----|
| Synthesis of a Panel of Carbon-13-Labeled Phosphosphingolipids | 65 |
|---|----|

Chapter 5

| | |
|---|----|
| Synthesis of 6-Hydroxysphingosine and Alpha-Hydroxy Ceramide using a Cross-Metathesis Strategy | 77 |
|---|----|

Chapter 6

| | |
|--|----|
| Synthesis of Ceramide-Mimetic Aziridines as Potential Mechanism-Based Enzyme Inhibitors | 93 |
|--|----|

Chapter 7

Summary and Future Prospects 105

List of Publications 123

Samenvatting 125

Curriculum Vitae 128

Chapter 1

Chemical Tools to Study Sphingolipid Metabolism

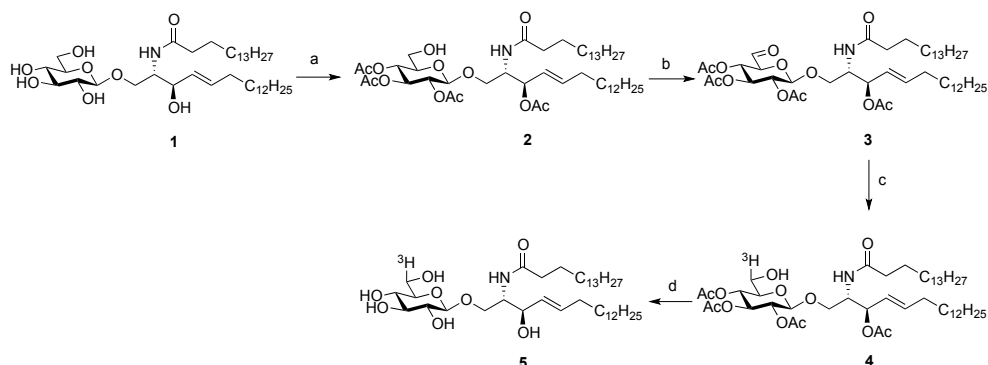
1.1 Introduction

Sphingolipids are, together with sterols and glycerophospholipids, the major lipids present in mammalian cell membranes. Sphingolipids are composed of ceramide that is functionalized at the primary alcohol with a hydrophilic head group which can be neutral or charged carbohydrates, phosphates or phosphodiesteres.^[1] Ceramide in turn is

composed of sphingosine, the secondary amine of which is condensed with a fatty acid to give the corresponding amide. Sphingolipids are found in the outer cell membrane layer, with the hydrophobic part embedded within the membrane and the hydrophilic part pointing towards the extracellular matrix. Sphingolipids are key players in many physiological processes and malfunctioning of sphingolipid metabolism is at the basis of many human pathologies. For these reasons, many studies have been directed towards understanding of sphingolipid biochemistry and biology: how are they (dis)assembled and what is the molecular basis of their physiological activity? In these studies, synthetic sphingolipid analogues and derivatives have been used. The main body of the work in this Thesis focuses on such sphingolipid analogues, and specifically on the design and synthesis of stable-isotope-encoded sphingolipids. This chapter provides a brief overview of the various classes of synthetic, labeled sphingolipids, including stable-isotope-encoded derivatives but also analogues bearing other chemical reporter groups (such as fluorophores and radio-isotopes).

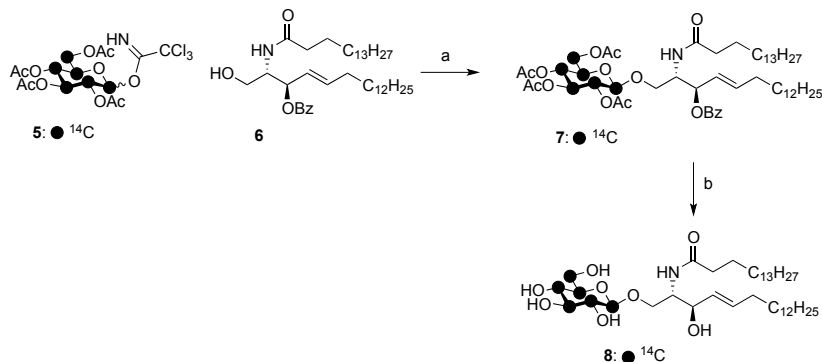
1.2 Radioisotope labeled sphingolipids

In the past decades, several strategies for the preparation of radio-isotopic sphingolipids have been reported. Carbon-14^[2-6] and tritium (hydrogen-3)^[7-17] are obvious choices with respect to the nature of the radioisotope employed and examples of both have appeared in the literature. The most commonly applied method for introducing carbon/hydrogen isotopes comprises *N*-acylation of sphingosine with an appropriately labeled fatty acid.^[2-4, 7,20] Other commonly used methods to introduce radioisotopes are reductive tritiation of the alkene bond in sphingosine (catalytic palladium on charcoal, $^3\text{H}_2$ (g))^[8,9], or by reducing the ketone of 3-ketosphingosine to sphingosine using sodium borotritide (NaB^3H_4).^[10-13] Radioisotopes can also be introduced to the carbohydrate moiety in glycosphingolipids. For instance, introduction of tritium (^3H) in the glucose moiety of glucosylceramide can be accomplished by selective manipulation of the C6-hydroxyl of glucosylceramide **1** as described in Scheme 1.1. Selective 6-O-tritylation, followed by global O-acetylation and removal of the trityl group using acidic conditions resulted in partially protected resulted glucosylceramide **2**. The free primary hydroxyl in **2** was oxidized to aldehyde **3** and next reduced with NaB^3H_4 (**3** to **4**) followed by global deprotection, yielding tritium-labeled glucosylceramide **5**.^[14] A similar strategy (selective oxidation followed by tritide reduction) has been developed for galactosyl/GM2/GalNAc^[15,16] sphingolipids, although here the primary alcohol-to-aldehyde transformation was effected by the use of galactose oxidase.

Scheme 1.1 Synthesis of tritium-labeled glucosylceramide **5**.

Reagents and conditions: (a) (i) TrCl , pyridine, 40 °C, 18 h; (ii) Ac_2O , pyridine, r.t., 20 h; (iii) HBr (33% in HOAc), HOAc/DCM (1:1), -10 °C, 5 min; (b) (i) N,N -dicyclohexylcarbodiimide, H_3PO_4 , DMSO ; (ii) HOAc ; (c) (i) NaB^3H_4 , 1 mM NaOH (aq), THF , r.t., 20 h; (ii) 1 M HOAc (aq); (d) NaOMe , MeOH , r.t., 20 h.

An alternative strategy for the introduction of radio-isotopic labels in the glycan part comprises glycosylating the primarily hydroxyl of the ceramide with a radio-isotope labeled glycan donor.^[5,6] This strategy is exemplified in the use of carbon-14 labeled glucosyl donor **5** (Scheme 1.2). Donor **5** was condensed with partially protected ceramide **6** producing glucosylceramide **7**, followed by global deprotection (sodium methoxide in methanol) resulting in ^{14}C -glucosylceramide **8**.^[6]

Scheme 1.2 Synthesis of ^{14}C -glucosylceramide.

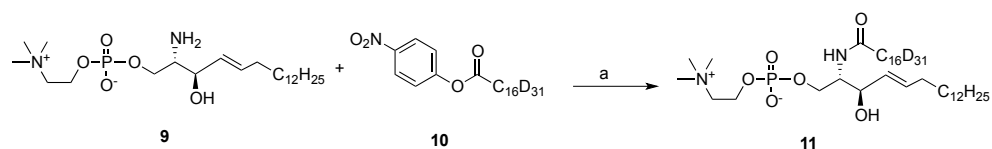
Reagents and conditions: (a) BF_3OEt_2 , DCM , -10 °C to r.t., 10 h, 55%; (b) NaOMe , MeOH , r.t., 24 h, 95%.

1.3 Stable isotope-labeled sphingolipids

Both carbon-13^[6,18,19] and deuterium (hydrogen-2)^[20,21] have been used for isotopic labeling of sphingolipids. The most convenient method for introducing deuterium isotopes

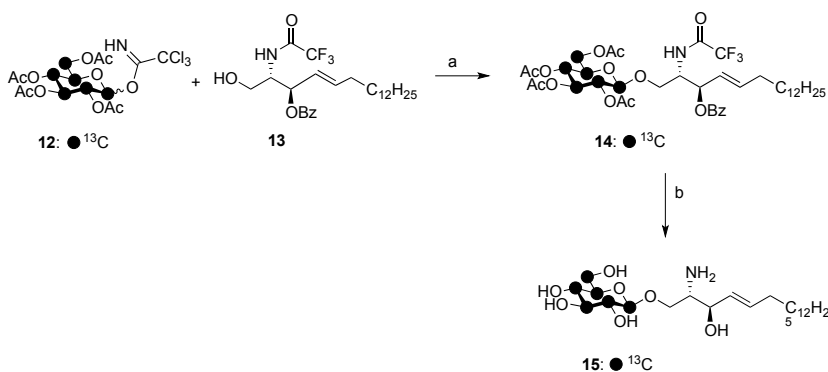
has been *N*-acylation of sphingosine with a deuterium labeled fatty acid.^[20] In the example depicted in Scheme 1.3, phosphocholanyl sphingosine **9** was *N*-acylated using deuterated nitrophenyl ester **10** with K_2CO_3 as the base, resulting in deuterated sphingomyelin **11**. This compound was used in NMR-experiments aimed to establish the conformation and interaction of sphingolipids with other lipids in membranes.^[20]

Scheme 1.3. Synthesis of deuterium labeled sphingomyelin **11**.



Reagents and conditions: (a) K_2CO_3 , DMF/DCM, r.t., 24 h.

Glycosphingolipids in which the carbohydrate moiety contains carbon-13 atoms have been synthesized as well. $^{13}C_6$ -Glucosylsphingosine **15**^[6] was for instance prepared (Scheme 1.4) by condensation of carbon-13 labeled glucosyl-donor **12** with *N*-trifluoroacetamide protected sphingosine **13** using BF_3OEt_2 as the activator for the glycosylation reaction. The resulting fully protected and carbon-13-labeled glucosylsphingosine **14** was transformed into **15** by treatment with sodium methoxide.



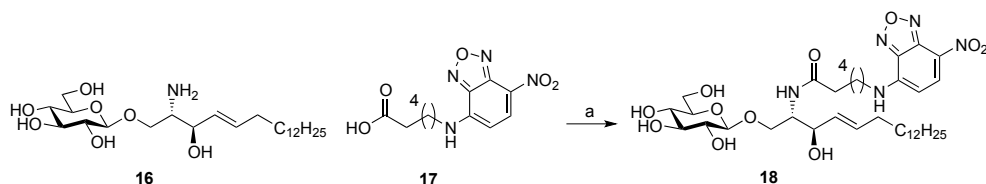
Reagents and conditions: (a) BF_3OEt_2 , DCM, $-10\text{ }^{\circ}C$ to r.t., 10 h, 27%; (b) NaOMe, MeOH, r.t., 24 h, 78%.

1.4 Fluorescent sphingolipids

Sphingolipids containing a fluorescent reporter group have been used over the past decades to follow the trafficking and localization of sphingolipids in living cells as well as to measure activities of several sphingolipid-processing enzymes. The most common fluorophore applied in these studies is 7-nitro-2-1,3-benzoxadiazole (NBD).^[7,22-25]

Alternative fluorophores are borondipyrromethene (BODIPY),^[26-31] pyrene,^[32-35] diphenylhexatrienyl,^[36] lissamine rhodamine,^[37] Nile red^[38] and dansyl.^[39] Again, *N*-acylation of sphingosine with an appropriately modified fatty acid to give the corresponding ceramide has been the method of choice. As an example (Scheme 1.5), the synthesis of NBD-glucosylceramide **18** by *N*-acylation of glucosylsphingosine **16** with NBD-hexanoic acid **17** under the agency of PPh_3 and 2,2-dipyridyldisulfide has been accomplished.^[22]

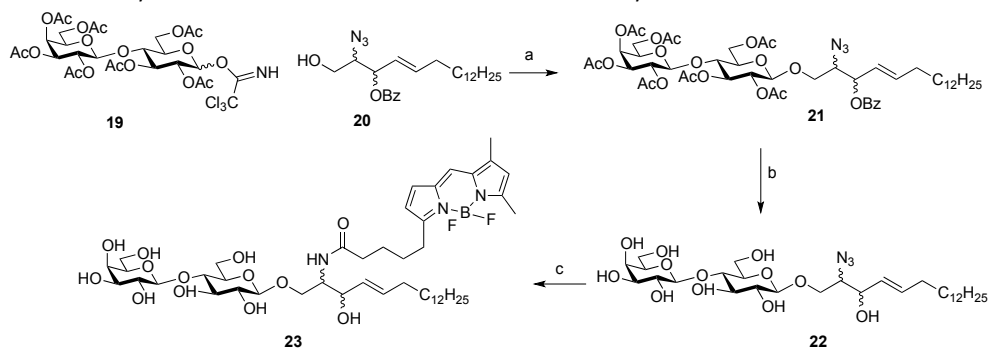
Scheme 1.5 *N*-acylation of glucosylsphingosine **16** with NBD-hexanoic acid **17**.



Reagents and conditions: (a) PPh_3 , 2,2 dipyridyldisulfide, DCM.

A related strategy had been applied by Bittman^[26], who prepared the three stereoisomeric (2*R*,3*R*), (2*S*,3*S*) and (2*R*,3*S*) lactosylceramides to study the influence of the stereochemistry of the sphingosine base on endocytosis (Scheme 1.6). In the first instance the stereoisomeric sphingosines **20** were synthesized, after which glycosylation of primary hydroxyl with donor lactoside **19** produced the fully protected lactosylsphingosines **21**. Removal of the ester protecting groups (sodium methoxide in methanol) gave the set of stereoisomers of azido-lactosylsphingosine **22**. Staudinger reduction of the azide in **21** and ensuing condensation of the *in situ* formed amine with BODIPY- $\text{C}_5\text{-NHS}$ provided target compounds **23**.

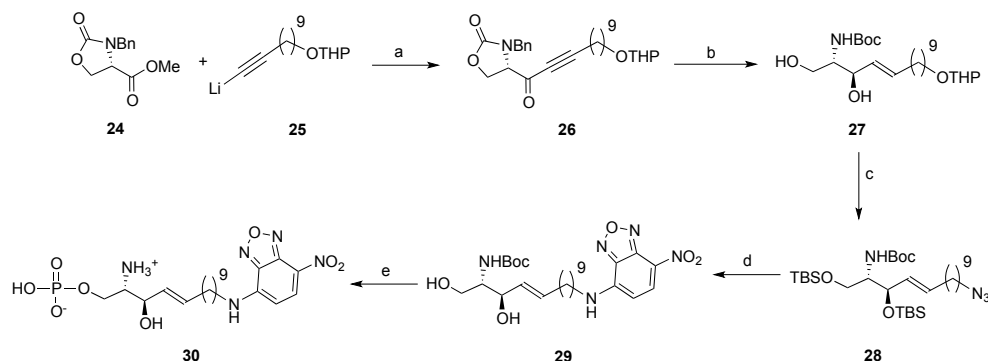
Scheme 1.6 Synthesis of BODIPY-labeled stereoisomers of lactosylceramide **23**.



Reagents and conditions: (a) BF_3OEt_2 , DCM, 20 h, (2*R*,3*R*) 66%, (2*R*,3*S*) 54%; (b) NaOMe, MeOH, 6 h, (2*R*,3*R*) 68%, (2*R*,3*S*) 62%; (c) BODIPY- $\text{C}_5\text{-NHS}$, PPh_3 , THF/ H_2O (9:1), (2*R*,3*R*) 38%, (2*R*,3*S*) 36%, (2*S*,3*S*) 38% (over three steps).

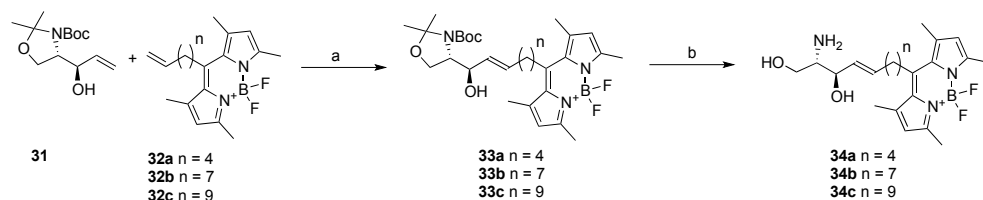
In the above examples, the fluorophores were all located on the fatty acid of the ceramide. In case however lysosphingolipids (that is, compounds featuring sphingosine, not ceramide) are the subject of study, the fluorescent label has to be introduced in the sphingosine backbone. One such strategy entails addition of lithium-alkyne **25** to ester **24** to form alkyne **26** (Scheme 1.7). Stereoselective reduction of the ketone in **26**, followed by Birch reduction of the triple bond to the trans-alkene, removal of the cyclic carbamate and *N*-benzyl and *N*-Boc protection of the thus liberated amine gave sphingosine **27**.^[25] Next, the hydroxyls of the *N*-Boc protected sphingosine **27** were silylated followed by removal of the THP protective group. The primary hydroxyl was converted to an azide, by mesylation followed by azide substitution to produce azido-sphingosine **28**. This azido-sphingosine **28**, after removal of all protecting groups, can be used as bioorthogonal handle (see part 1.6) for metabolic studies of sphingolipids. The azide in **28** was reduced to the amine, which was then reacted with NBD-Cl in a nucleophilic aromatic substitution reaction to give **29**. The silyl-groups of the protected NBD-sphingosine were removed using 2 M aqueous hydrochloric acid. The resulting NBD-sphingosine **29** was next phosphorylated to produce NBD-sphingosine-1-phosphate **30**.

Scheme 1.7 Synthesis of NBD-labeled sphingosine-1-phosphate **30**.



Reagents and conditions: (a) *n*-BuLi, THF, -100 °C, 93% (b) (i) diisobutylaluminum hydride, 2,6-di-*tert*-butyl-4-methylphenoxide, toluene, 0 °C, 99%; (ii) Li, NH₃, THF, reflux, 96%; (iii) KOH, EtOH, reflux; (iv) Boc₂O, K₂CO₃, THF, H₂O, 0 °C, 84%; (c) (i) TBSCl, imidazole, DMF; (ii) MgBr₂, Et₂O, 82% (over the two steps); (iii) MsCl, Et₃N, THF; (iv) NaN₃, DMF, 50 °C, 89% (for two steps); (d) (i) PPh₃, 10% aq THF, 60 °C; (ii) NBDCl, Et₃N, THF; (iii) 2 M HCl, MeOH, 82% (over the three steps); (e) (i) CBr₄, P(OMe)₃, pyridine, -10 °C, 82%; (ii) TMSBr, CH₃CN, 71%.

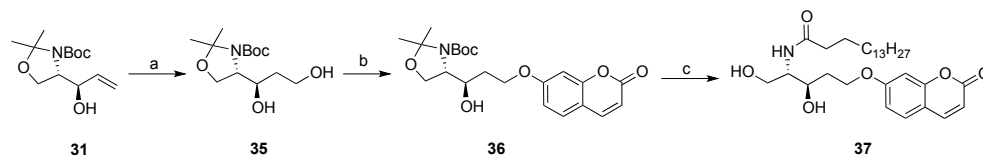
Cross-metathesis has often been used as a versatile strategy to create chemically modified sphingosines.^[40,41] For instance, alkenes **31** and **32** undergo efficient cross-metathesis using Grubbs' 2nd catalyst to give, after deprotection, fluorescent sphingosine derivative **34** (Scheme 1.8).

Scheme 1.8 Synthesis of BODIPY-sphingosine **34** featuring cross-metathesis as the key step.

Reagents and conditions: (a) Grubbs' 2nd catalyst, DCM, reflux, **31a**: 67%, **31b**: 79%, **31c**: 57%; (b) HCl, dioxane, **31a**: 48%, **31b**: 68%, **31c**: 54%.

1.5 Fluorogenic ceramides

Bedia and co-workers have developed compound **37** as a fluorogenic substrate to measure ceramidase activities.^[42] After ceramidase-mediated deacylation, the sphingosine is subjected to oxidation and subsequent beta-elimination releasing umbelliferone, which becomes fluorescent in alkaline conditions. The amount of fluorescent signal produced is proportional to the activity of ceramidase. The synthesis of **37** is depicted in Scheme 1.9 and comprises hydroboration and subsequent oxidation of the alkene in **31** followed by mesylation of the primary alcohol in **35**, nucleophilic substitution of the resulting mesylate, acid-mediated cleavage of the Boc- and isopropylidene protective groups and final *N*-acylation (**36** to **37**).

Scheme 1.9 Synthesis of umbelliferone-ceramide **37**.

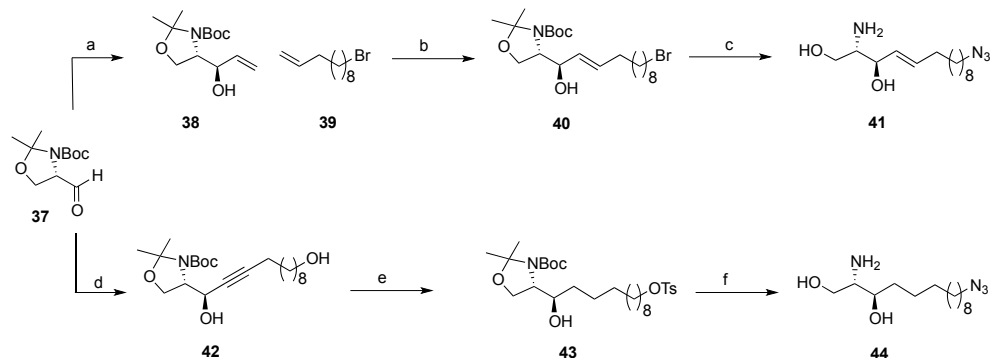
Reagents and conditions: (a) (i) BH₃, THF, 0 °C to r.t., 4 h; (ii) H₂O₂, 80%; (b) (i) MsCl, Et₃N, DCM, 0 °C, 30 min; (ii) umbelliferone, CsCO₃, acetone, 65 °C, 6 h; (c) (i) TFA, H₂O, DCM, 0 °C, 10 min; (ii) palmitoyl chloride, NaOAc, H₂O, DCM, 0 °C to r.t., 16 h.

1.6 Bioorthogonal sphingolipids.

Sphingolipid derivatives containing an azide or terminal alkyne have been applied in bioorthogonal chemistry based studies.^[43-46] The main advantages of bioorthogonal groups is that they are small and therefore are less likely to interfere with, or prohibit, sphingolipid metabolic steps. Fluorophores, biotin or mass tags can be introduced in a later stage by means of click chemistry. Scheme 1.10 provides representative examples of bioorthogonal sphingolipids by depicting the syntheses of azido-sphingosine **41** and azido-sphinganine **45**.^[43] Delgado and co-workers reacted alkene **38** and bromo-alkene **39** in a cross-metathesis event to produce bromo-sphingosine **40**. Substitution of the bromide for azide followed by global deprotection gave azido-sphingosine **41**. In a conceptually

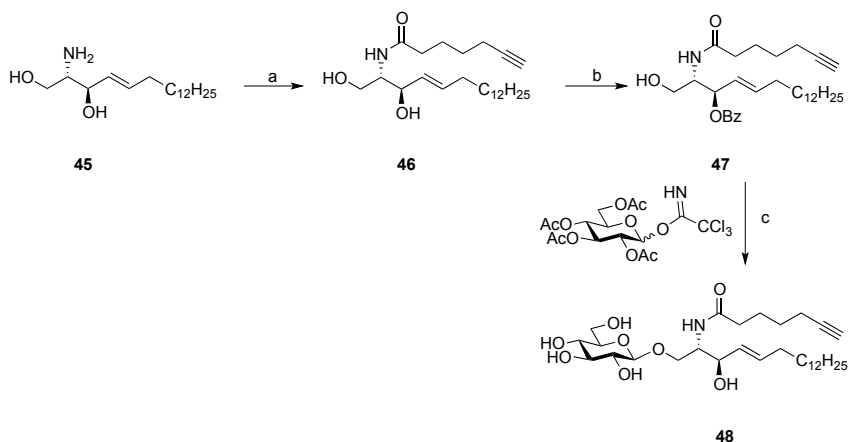
different strategy, Garner aldehyde **37** was reacted with the lithium anion of undec-10-yn-1-ol to give alkyne **42**. Full reduction of the alkyne in **42** followed by introduction of the azide (tosylation of the primary free alcohol followed by nucleophilic displacement of the resulting tosylate) and removal of the acid-labile groups gave azidosphinganine **44**.

Scheme 1.10 Synthesis of azido-sphingosine **41** and azido-sphinganine **44**.



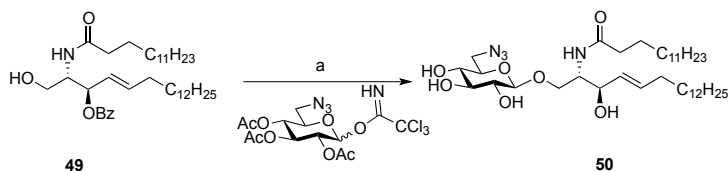
Reagents and conditions: (a) (i) Tetravinyl tin, THF, *n*-BuLi, -78 °C to r.t., 1h; (ii) **37**, THF, -78 °C to r.t., 20 h, 49%; (b) Grubbs' 2nd generation catalyst, DCM, reflux, 59%; (c) (i) NaN₃, DMF, 80 °C, 93%; (ii) HCl, MeOH, r.t., 1 h, 84%; (d), undec-10-yn-1-ol, BuLi, HMPA, THF, -78 °C, 50%, (e) (i) H₂ (g), Rh catalyst, MeOH, 89%; (ii) TsCl, DMAP, Et₃N, DCM, r.t., 58%; (f) (i) NaN₃, DMF, 80 °C, 76%; (ii) HCl, MeOH, r.t., 1 h, 85%.

Besides introduction of a bioorthogonal tag in the sphingosine/sphinganine base, such moieties can also be introduced in the fatty acid moiety of the corresponding ceramide, or alternatively in the carbohydrate moiety of glycosphingolipids. Scheme 1.11^[44] represents an example of the former in the synthesis of alkyne-terminating glucosylceramide **48** following established procedures both for the N-acylation and O-glycosylation steps.

Scheme 1.11 Synthesis of *N*-alkyne glucosylceramide **48**.

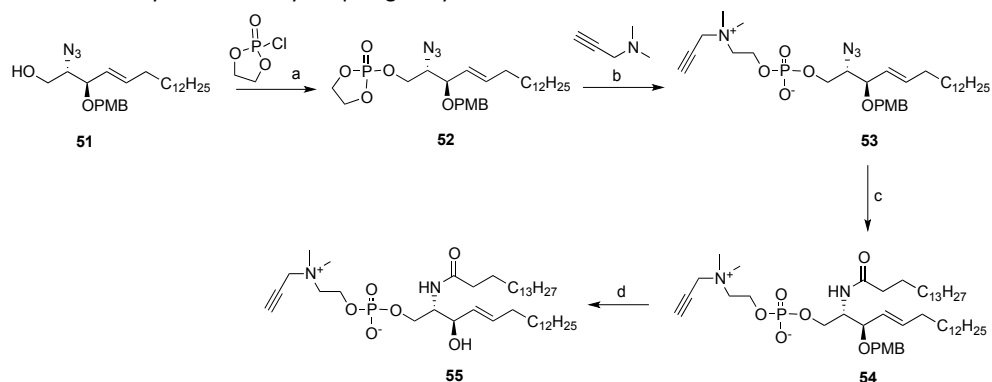
Reagents and conditions: (a) 7-heptynoic acid *N,O*-succinate ester, Et₃N, DCM, r.t., 16 h; (b) (i) TBDMSCl, imidazole, DCM, r.t., 20 h, 60% (two steps); (ii) BzCl, pyridine, r.t., 2 h, 95%, (iii) HF.pyridine, THF, r.t., 16 h, 50%; (c) (i) BF₃OEt₂, DCM, r.t., 2.5 h, 44%; (ii) NaOMe, MeOH, r.t. 16 h; 71%.

Scheme 1.12 depicts an illustrative example of a synthesis of bioorthogonal glucosylceramide **50**,^[44] with the bioorthogonal tag now on the sugar moiety, and again by following standard transformations related to those described earlier in this Chapter.

Scheme 1.12. Synthesis of 6-deoxy-6-azido glucosylceramide.

Reagents and conditions: (a) BF₃OEt₂, DCM, r.t., 2.5 h, 20%; (ii) NaOMe, MeOH, r.t. 16 h, 48%.

Finally, with respect to bioorthogonal sphingosines, alkyne-modified sphingomyelin **55** has been synthesized^[45] from partially protected sphingosine **51** as shown in Scheme 1.13. Treatment of **51** with cyclic chlorophosphate and base resulted in the formation of phosphate triester **52**. Treatment of triester **52** with propargyldimethylamine and Lewis acid led to the quaternary ammonium salt **53**. Reduction of the azide in **53**, ensuing *N*-acylation followed by removal of the *para*-methoxybenzyl group gave sphingomyelin derivative **55**.

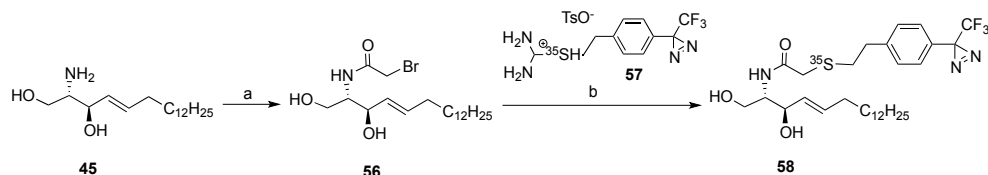
Scheme 1.13 Synthesis of alkyne-sphingomeylin **55**.

Reagents and conditions: (a) Et₃N, DMAP, toluene, 12 h; (b) TMSOTf, DCM, 12 h, 70% (for two steps); (c) (i) Zn, HOAc, 24 h; (ii) NHS-palmitate, DMAP (cat), DCM, 12 h; (d) TFA, DCM, 0 °C, 1 h, 76% (for two steps).

1.7 Photo-reactive sphingolipids

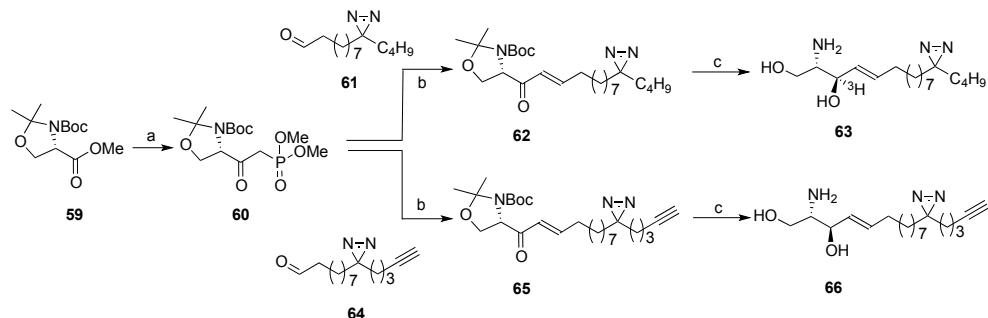
The previous parts described chemical tools that mainly focused on trafficking, localization and quantification of sphingolipids and their metabolic pathways. However, these tools do not allow the identification of proteins or other biomolecules that may interact with specific sphingolipids. With the aim to enable the study of protein-sphingolipid interactions, diazirine sphingolipids have been developed.^[47-52] Under influence of UV-light, diazirines collapse under expulsion of dinitrogen to yield a carbene that will insert in any X-H bond available. When the diazirine is in close proximity to a sphingolipid-interacting protein a covalent linkage will be the result. For the purpose to identify such sphingolipid-interacting proteins, the development of diazirine-sphingolipids further functionalized with radio-isotopes^[47-49,52] or bioorthogonal handles,^[50] have been described.

Scheme 1.14 depicts the synthesis of ³⁵S-tagged diazirine-containing sphingosine derivative **58**, as a relevant example of the former class.^[46] *N*-Bromoacetylation of sphingosine **45** yielded alkylating agent **56**, which was reacted with diazirine reagent **57** and base to give target compound **58**.

Scheme 1.14 Synthesis of *N*-acyl-[³⁵S]-diazirine ceramide **58**.

Reagents and conditions: (a) 2-bromoacetyl chloride, Et₃N, MeOH, -40 °C to r.t., 1 h, 91%; (b) **57**, K₂CO₃, MeOH, 55-70 °C; 39%.

An alternative sphingosine-based photo-cross linker, now with the diazirine embedded within the sphingosine backbone, rather than the fatty acid moiety, and bearing a tritium atom, is represented by compound **63**, the synthesis of which is depicted in Scheme 1.15. Ester **59** was reacted with dimethoxymethyl phosphonate forming ketophosphonate **60**.^[48] Horner-Wadsworth-Emmons olefination of aldehyde **61** with ketophosphonate **60** provided protected 3-keto-sphingosine **62**, which was reduced with NaB³H₄, followed by acidic removal of the isopropylidene and *N*-Boc protective groups yielding **63**. More recently, variation of this synthesis strategy enabled the synthesis of compound **66** (Scheme 1.15), now bearing a bioorthogonal alkyne instead of a radioisotope as a reporter entity.

Scheme 1.15 Synthesis of tritium-diazirine-sphingosine **63** and alkyne-diazirine-sphingosine **66**.

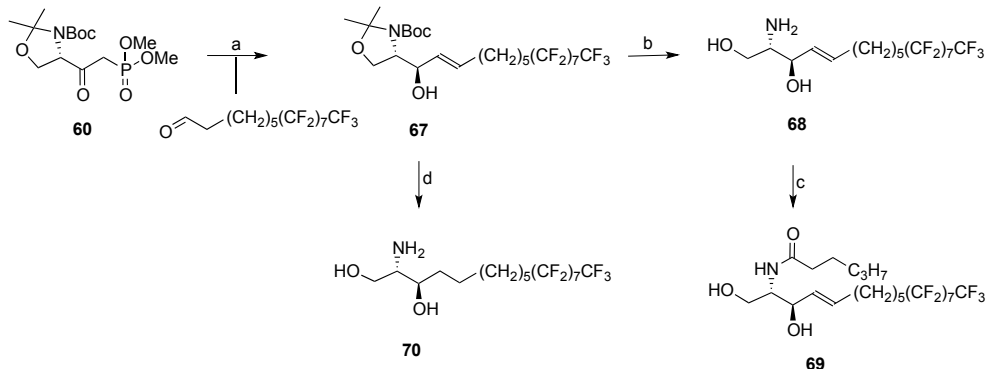
Reagents and conditions: (a) (i) CH₃PO(OMe)₂, BuLi, THF, -78 °C, 30 min; (ii) **59**, -78 °C, 30 min; (iii) 10% citric acid (aq); (b) (i) K₂CO₃, H₂O, CH₃CN, 5 min; (ii) **61** or **64**, DCM, 45 °C, 6 h; (c) (i) NaBH₄/NaB³H₄, CeCl₃·H₂O, MeOH, 4 °C, 3 h; (ii) 6 M HCl, MeOH, 50 °C, 6 h.

1.8 Fluorine-containing sphingolipids

In a recent article, Saito and co-workers describe the synthesis and use of fluorine containing sphingolipids for metabolic studies.^[53] Fluorine is considered to be an attractive hydrogen isostere at least when regarding steric bulk: fluorine and hydrogen substituents on carbon have about the same size. In case, numerous fluorines are installed, as in the described study, fluorous phase extraction should enable enrichment of fluorine-containing compounds, including metabolites derived from the parent compounds, from

lipid fractions. The synthesis of fluororous sphingolipids **68** and **69** and fluororous ceramide **69** are depicted in Scheme 1.16 and followed a synthetic strategy closely related to that described above for the synthesis of photo-reactive diazirine sphingolipids (see part 1.7), featuring a Horner-Wadsworth-Emmons (HWE) reaction as the key step.

Scheme 1.16 Synthesis of fluorine-containing sphingolipids.



Reagent and conditions: (a) (i) K_2CO_3 , H_2O , THF, $0\text{ }^\circ\text{C}$ to r.t., 72 h, 79%; (ii) $\text{Zn}(\text{BH}_4)$, Et_2O , -78 to $0\text{ }^\circ\text{C}$, 88%; (b) 1 M HCl, THF, $70\text{ }^\circ\text{C}$, 18 h, 85%; (c) hexanoyl chloride, NaOAc, THF/ H_2O , r.t., 12 h, 90%; (d) (i) Pd/C, H_2 (g), MeOH, r.t., 3 h; (ii) 1 M HCl, THF, reflux, 2 h, 63%.

1.9 Contents of this thesis

This first Chapter provides an overview of the chemical tools that have been developed for studying sphingolipids, with as main focus the routes of synthesis. The described research tools and the routes of synthesis are complementary to the reagents and chemistry described in this Thesis, which, as mentioned before, focused on the design and development of carbon-13-enriched sphingolipid derivatives. **Chapter 2** describes the synthesis of a panel of carbon-13-labeled (glyco)sphingolipids containing five carbon-13 isotopes in the sphingosine backbone. **Chapter 3** discusses optimization of the glycosylation reaction between glycosyl donors and protected sphingosines. **Chapter 4** describes the synthesis of a panel of carbon-13-labeled phosphosphingolipids. **Chapter 5** describes the synthesis of another sphingosine backbone, namely 6-hydroxysphingosine, which was assembled using a cross-metathesis strategy. **Chapter 6** discusses the synthesis of modified sphingolipids, containing an aziridine for activity based protein profiling. **Chapter 7** provides a summary of the results described in this Thesis and projects some directions for future research.

1.10 References

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Chapter 2

Synthesis of a Panel of Carbon-13-Labeled (Glyco)Sphingolipids

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2.1 Introduction

Sphingolipids and their derivatives (glycosphingolipids, phosphosphingolipids, sphingomyelins) are important structural components of mammalian cell membranes. The biosynthesis of sphingolipids is a tightly controlled process, and disruption of a specific metabolic step can lead to disease. A variety of genetic disorders linked to sphingolipid metabolism occur in man. Often, these diseases are characterized by mutations in genes

that encode for enzymes or chaperones involved in a specific metabolic step in the lysosomal degradation of sphingolipids. Prominent examples of such lysosomal storage disorders are Gaucher disease (inherited defect in acid glucocerebrosidase, GBA1, the enzyme responsible for the hydrolysis of glucosylceramide to glucose and ceramide) and Fabry disease (inherited defect in lysosomal α -galactosidase, the enzyme responsible for the hydrolysis of globotriaosylceramide to galactose and lactosylceramide).^[1]

Studies on Gaucher and Fabry diseases revealed that both are characterized by storage of the substrate of the genetically impaired enzyme (i.e., glucosylceramide in Gaucher and globotriaosylceramide in Fabry), but also the occurrence of alternative metabolic pathways.^[1–3] There is also evidence that metabolites produced by these alternative pathways, lysoglycosphingolipids in both cases, may be involved in, or are perhaps even causative in, the onset and development of the disease.^[5] Those discoveries were made thanks in part to stable-isotope-labeled ($^{13}\text{C}_5$) sphingolipids, which were synthesized for this purpose. These studies led to the realization that a comprehensive set of sphingolipids differing both in structure and in the number of ^{13}C -atoms embedded in both the sphingosine and the *N*-acyl (palmitate) moieties, as represented by the general structure in the insert of Figure 2.1, would be a very useful set of research tools.

Some relevant sphingolipid biosynthesis pathways are shown in Figure 2.1.^[4] At the basis of the biosynthesis of all sphingolipids is sphinganine **1**, itself the condensation product of serine and palmitate. In a reaction catalyzed by sphinganine acyl transferase (SAT), the free amine in **1** is condensed with a fatty acid, here shown as palmitate but in reality one of a number of saturated or partially unsaturated fatty acids of varying size. In the next step, the resulting dihydroceramide (**2**) is dehydrogenated through the action of dihydroceramide dehydrogenase (DCD) to produce ceramide **3**. At this stage, a number of different pathways can take place, giving rise to a wide variety of sphingolipids featuring different polar head groups. Glucosylceramide (**4**) is the product of the glucosylceramide synthase (GCS) catalyzed condensation of **3** with UDP-glucose. Glucosylceramide (**4**) in turn is the starting point for the synthesis of a wide variety of glycosphingolipids and gangliosides featuring oligosaccharides of different sizes and natures, and including branched oligosaccharides. After its synthesis, glucosylceramide is modified to more complex glycosphingolipids by the sequential action of glycosyltransferases. As a representative example, globotriaosylceramide (**5**) emerges after sequential β -galactosylation and α -galactosylation of glucosylceramide (**4**) effected by two independent glycosyltransferases.^[4] In time, sphingolipids are internalized by endocytosis, and transported to the lysosomal compartments, where they are degraded. The degradation of glycosphingolipids is commonly viewed to take place in a stepwise manner, with the product of one enzyme acting as the substrate of the next enzyme of the disassembly line. In this fashion, globotriaosylceramide (**5**) is transformed by the action of lysosomal α -

galactosidase into lactosylceramide. Lysosomal β -galactosidase next removes the β -galactose residue to deliver glucosylceramide, which in turn is deglycosylated by GBA1 to give ceramide as the penultimate degradation product. Finally, acid ceramidase (ACase) hydrolyses the amide bond to produce sphingosine (**6**; Figure 2.1) and palmitate for reuptake into the cytoplasm as new building blocks for catabolism.

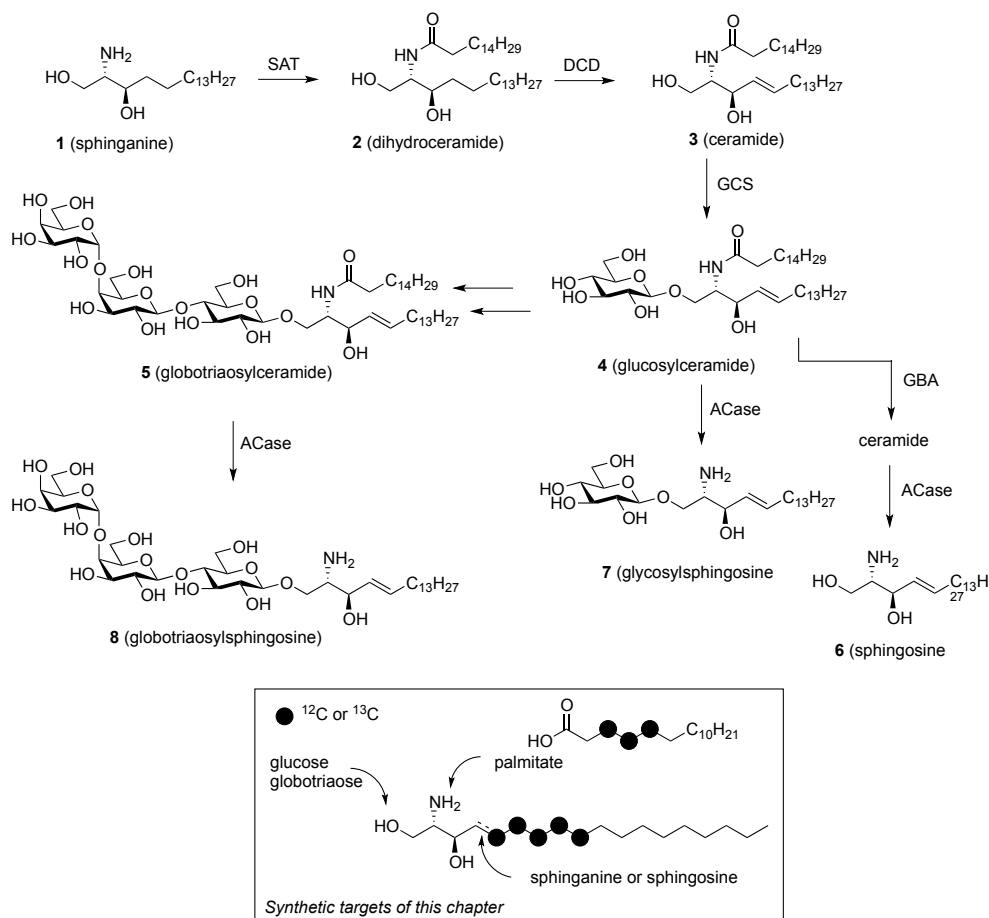


Figure 2.1 Partial overview of sphingolipid metabolism in man, and the target structures (insert) of the synthetic studies presented here. ACase: acid ceramidase; DCD: dihydroceramide dehydrogenase; GBA: glucocerebrosidase; GCS; glucosylceramide synthase; SAT: sphinganine acyl transferase.

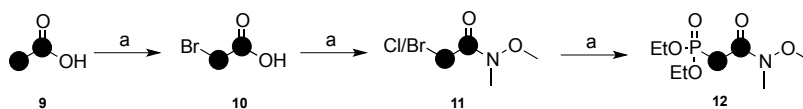
In contrast to common belief, it was found a few years ago that in tissue from Fabry patients, as well as in animal models, which are characterized by elevated levels of globotriaosylceramide due to genetically and partially disabled lysosomal α -galactosidase, the *N*-acyl chain of a portion of the accumulated globotriaosylsphingosine is removed, resulting in the formation of the lysoglycosphingolipid, globotriaosylsphingosine (**8**).^[2] A

related alternative metabolic pathway also appeared to occur in Gaucher patients: accumulated glucosylceramide, caused by partially dysfunctional GBA1, is partially deacylated to produce glucosylsphingosine (**7**).^[3] These alternative pathways are probably occurring through the action of acid ceramidase (ACase), although this needs to be confirmed. The generation of stable-isotope-labeled [¹³C₅]-globotriaosylsphingosine (**8**) and glucosylsphingosine (**7**) allows the detailed study of such alternative metabolic pathways. Stable-isotope analogues are also very useful for the diagnosis of both diseases and for monitoring their treatment, with corrections for glycolipid metabolism being reflected by lowered levels of lysolipids in tissue samples.^[5–7] With this reasoning in mind, the idea came to construct a focused library of stable-isotope (glyco)sphingosine and (glyco)sphingolipid derivatives. In the design, it was decided to incorporate five ¹³C-atoms into the sphingosine base, and three into the palmitate, to obtain compounds that would be easily detected, together with their unlabeled counterparts, from complex biological lipid fractions. The details of their synthesis, relying on a cross-metathesis reaction to give stable-isotope-labeled sphingosine for further elaboration into a library of 24 compounds, are reported here.

3.1 Results and discussion

Ready access to [¹³C₅]-sphingosine, the common backbone of all target structures, is crucial to the synthesis of the panel of [¹³C_n]-sphingolipids. Based on literature precedence,^[8,9] cross-metathesis of [¹³C₅]-pentadeca-1-ene (**20**) with aminodiol **21** was selected as the key step towards this common intermediate.^[10–16] Introduction of carbon-13 isotopes into **20** was achieved using [¹³C]-potassium cyanide and [¹³C₂]-acetic acid, the latter of which was converted into Horner–Wadsworth–Emmons (HWE) reagent **12** in a four-step procedure as shown in Scheme 2.1. Transformation of acetic acid **9** into bromoacetic acid **10** by a Hell-Volhard-Zelinsky reaction^[8] was followed by treatment of **10** with oxalyl chloride and addition of *N,O*-dimethylhydroxylamine in an one-pot fashion to give a mixture of bromo- and chloro-*N*-methoxy-*N*-methylacetamides (**11**). Subjection of this mixture of Weinreb amides to Arbuzov reaction conditions gave the target HWE reagent (**12**) in 74% yield over four steps.

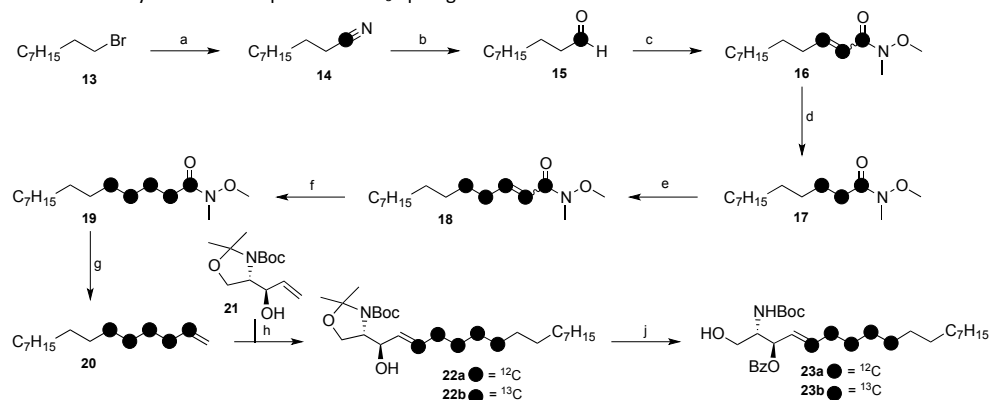
Scheme 1. Synthesis of the ¹³C₂-Horner-Wadsworth-Emmons reagent **12**.



Reagents and conditions: (a) (i) TFAA (trifluoroacetic acid anhydride), Br₂, r.t., 20 h; (ii) water, 88 %; (b) (i) oxalyl chloride, DMF, CH₂Cl₂, 0 °C to r.t., 2 h; (ii) *N,O*-dimethylhydroxylamine, -78 °C to r.t., 2 h, 97 %; (c) triethylphosphite, 150 °C, 3 h, 95 %.

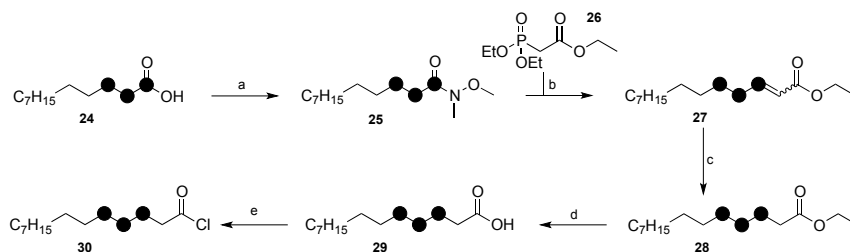
Next, 1-bromononane (**13**) was treated with [^{13}C]-potassium cyanide to give nitrile **14**, which was partially reduced to aldehyde **15** using DIBAL-H (diisobutylaluminium hydride) (87% over two steps; Scheme 2.2). This aldehyde was treated with reagent **12** and *n*-BuLi to give unsaturated [$^{13}\text{C}_3$]-Weinreb amide **16**, the C=C double bond in which was reduced to give **17** in 82% yield. A similar sequence of events, reduction of the Weinreb amide in **17** to the aldehyde, followed by HWE olefination with **12**, and C=C reduction, provided the corresponding Weinreb amide (**19**), which was transformed in two steps (reduction to the aldehyde, followed by Wittig reaction with *in situ* generated $\text{Ph}_3\text{P}=\text{CH}_2$) into [$^{13}\text{C}_5$]-pentadeca-1-ene (**20**) in 93% yield.

With [$^{13}\text{C}_5$]-pentadeca-1-ene (**20**) in hand, its cross-metathesis with alkene **21** under the conditions advocated in the literature (Grubbs 2nd generation catalyst, dichloromethane, **20:21** = 1:2) was investigated.^[9] Close examination of the metathesis product revealed partial elimination of one or two methylene units, leading to truncated cross-metathesis products. This came as a surprise, since there are several literature reports that describe the synthesis of unlabeled sphingosine using essentially the same procedure as described here, and none of these report the formation of truncated (C17 or C16) sphingosines.^[11–16] Methylene eliminations have been reported as side-reactions in (cross)-metathesis studies unrelated to the synthesis of sphingosine. These events are thought to be the result of alkene isomerization of terminal alkenes while bound to the ruthenium metal center.^[17–19] This isomerization can be prevented by the addition of acetic acid to the cross-metathesis reaction mixture.^[20] Indeed, the addition of acetic acid (20 mol% relative to **21**) to an otherwise unchanged reaction mixture led to a clean cross-metathesis reaction to give **22** as the major product in 81% yield. Sphingosine **22** was transformed into a suitable substrate for the ensuing glycosylation by protecting group manipulations. Benzoylation of the secondary alcohol in **22** and removal of the isopropylidene with a catalytic amount of *p*-TsOH in methanol/ethanol to suppress unwanted Boc (*tert*-butoxycarbonyl) cleavage led to the isolation of the key building block **23**.

Scheme 2.2 Synthesis of the protected $^{13}\text{C}_5$ -sphingosine **23**.

Reagents and conditions: (a) K^{13}CN , $\text{EtOH}/\text{H}_2\text{O}$, 80°C , 20 h, 95%; (b) DIBAL-H, THF, 0°C to r.t., 2.5 h, acidic work up, 92%; (c) (i) **12**, $n\text{-BuLi}$, THF, 0°C , 10 min; (ii) $^{13}\text{C}_1$ -decanal (**15**), THF, 0°C to r.t., 20 h, 87%; (d) Pd/C, H_2 (g), EtOAc, r.t., 20 h, 82%; (e) LiAlH_4 , THF, 0°C , 45 min, to give crude $^{13}\text{C}_3$ -dodecanal, which was added to a solution of (**12**, $n\text{-BuLi}$, THF, 0°C , 10 min), 0°C to r.t., 20 h, 77%; (f) Pd/C, H_2 (g), EtOAc, 93%; (g) LiAlH_4 , THF, 0°C , 45 min, then transfer to a solution of (MePh_3PBr , $n\text{-BuLi}$, THF, 0°C , 10 min), 0°C to r.t., 20 h, 93%; (h) **21**, Grubbs 2nd catalyst, AcOH, CH_2Cl_2 , reflux, 48 h, 81%; (j) (i) BzCl , DMAP, CH_2Cl_2 /pyridine, r.t., 20 h, 92%; (ii) MeOH/EtOH, $p\text{-TsOH}$, r.t., 20 h, 63%.

$^{13}\text{C}_3$ -Palmitoyl chloride **30** was obtained starting from commercially available $^{13}\text{C}_3$ -myristic acid (**24**; Scheme 2.3). Labeled acid **24** was converted into the corresponding Weinreb amide (**25**) by treatment with oxalyl chloride, and subsequent addition of N,O -dimethylhydroxylamine. The two-carbon elongation of **25** to give **27** was realized by reduction with DIBAL-H, and subsequent subjection of the resulting aldehyde to HWE-olefination with reagent **26**. Reduction of the double bond in **27**, saponification, and treatment with oxalyl chloride gave $^{13}\text{C}_3$ -palmitoyl chloride **30**.

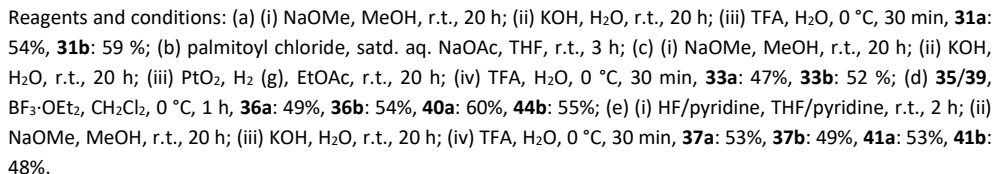
Scheme 2.3 Synthesis of $^{13}\text{C}_3$ -palmitoyl chloride **30**.

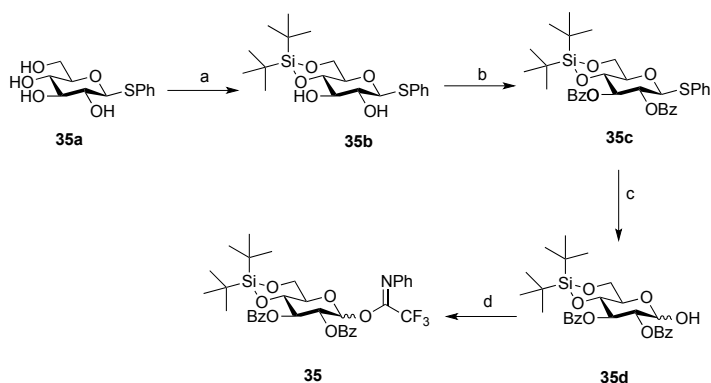
Reagents and conditions: (a) (i) oxalyl chloride, DMF, CH_2Cl_2 , 0°C to r.t., 2 h; (ii) N,O -dimethylhydroxylamine, -78°C to r.t., 2 h, 98%; (b) DIBAL-H, THF, -78°C , 30 min, to give crude $^{13}\text{C}_3$ -tetradecanal, which was added to a solution of (**26**, $n\text{-BuLi}$, THF, 0°C , 10 min), 0°C to r.t., 20 h, 81%; (c) Pd/C, H_2 (g), EtOAc, 20 h, 95%; (d) LiOH, THF/EtOH/ H_2O , 20 h, 95%; (e) oxalyl chloride, DMF, CH_2Cl_2 , 0°C to r.t., 2 h, 100%.

The synthesis of sphingolipids and glycosphingolipids in various ^{13}C -enriched forms based on **23** is shown in Scheme 2.4. Debenzoylation of **23b** with sodium methoxide in methanol,

followed by TFA (trifluoroacetic acid) mediated removal of the Boc group provided [$^{13}\text{C}_5$]-sphingosine (**31b**; 59% yield). Both [$^{13}\text{C}_0$]-**31a** and [$^{13}\text{C}_5$]-**31b** were condensed with either [$^{13}\text{C}_0$]-palmitoyl chloride or [$^{13}\text{C}_3$]-palmitoyl chloride **30** to give the panel of labeled ceramides **32a–32d**. Alternatively, debenzoylation of **23a/b**, reduction of the alkene moiety with Adams catalyst, and TFA-mediated Boc removal gave stable-isotope sphinganine pair **33a** and **33b**, which were used as starting materials to produce dihydroceramides **34a–34d**.

The glycosylated sphingolipids were assembled by reacting the labeled sphingosine alcohols with the appropriate glycosyl donors. Thus, *N*-phenyltrifluoroacetimidate glucose **35** (see Experimental Section for its synthesis; Scheme 2.5) and sphingosine **23a/b** were condensed in a reaction promoted by boron trifluoride diethyl etherate to give fully protected glucosylsphingosines **36a/b**. The moderate yield of the glycosylation reaction can be explained by the concomitant cleavage of the Boc group, which took place under the Lewis acidic reaction conditions. Glucosylation of **23a/b** using the corresponding perbenzoylated *N*-phenyltrifluoroacetimidate donor and boron trifluoride diethyl etherate was unproductive, and led only to the isolation of the product of Boc removal from **23a/b**. Global deprotection of **36** by successive treatment with HF/pyridine, sodium methoxide, and trifluoroacetic acid provided stable-isotope glucosylsphingosine pair **37a/b**. Both [$^{13}\text{C}_0$]-glucosylsphingosine (**37a**) and [$^{13}\text{C}_5$]-glucosylsphingosine (**37b**) were condensed with either [$^{13}\text{C}_0$]-palmitoyl chloride or [$^{13}\text{C}_3$]-palmitoyl chloride **30** to give the panel of labeled glucosylceramide derivatives **38a–38d**.



Scheme 2.5 Synthesis of donor glucoside **35**.


Reagents and conditions: (a) $t\text{Bu}_2\text{SiOTf}_2$, pyridine, DMF, $-40\text{ }^\circ\text{C}$, 30 min, 77 %; (b) BzCl , pyridine, r.t., 3 h, 98%; (c) NIS, TFA, CH_2Cl_2 , $0\text{ }^\circ\text{C}$, 3 h, 98%; (d) $\text{ClC}(\text{NPh})\text{CF}_3$, CsCO_3 , acetone, $0\text{ }^\circ\text{C}$, 2 h, 80 %.

Finally, the syntheses of globotriaosylsphingosines **41a/b** and globotriaosylceramides **42a–42d** were preformed. To this end, sphingosine **23** was condensed with trisaccharide donor **39**^[21] in a reaction promoted by boron trifluoride diethyl etherate to give fully protected globotriaosylsphingosines **40a/b**. Subsequent global deprotection by the same procedure as described above gave **41a/b**. Standard palmitoylation with either [$^{13}\text{C}_0$]-palmitoyl chloride or [$^{13}\text{C}_3$]-palmitoyl chloride gave the panel of globotriaosylceramides **42a–42d** to complete the library of labeled (glyco)sphingolipids.

The physical properties of all the labeled compounds matched those of their ^{12}C -counterparts, apart from their mass spectra and their ^1H and ^{13}C NMR spectra. As a representative example, Figure 2 shows the ^1H and ^{13}C NMR spectra of $^{13}\text{C}_5$ -globotriaosylsphingosine **41b** (Figure 2a, b and d), and the ^{13}C NMR spectrum of its non-enriched counterpart **41a** (Figure 2c). In Figure 2b, the ^{13}C -decoupled ^1H NMR spectrum of ^{13}C -labeled **41b** is shown, which is identical in all respects to the spectrum of unlabeled **41a**. Integration of the peaks due to the ^{13}C -labels in **41b** clearly shows the ratio of the incorporated atoms.

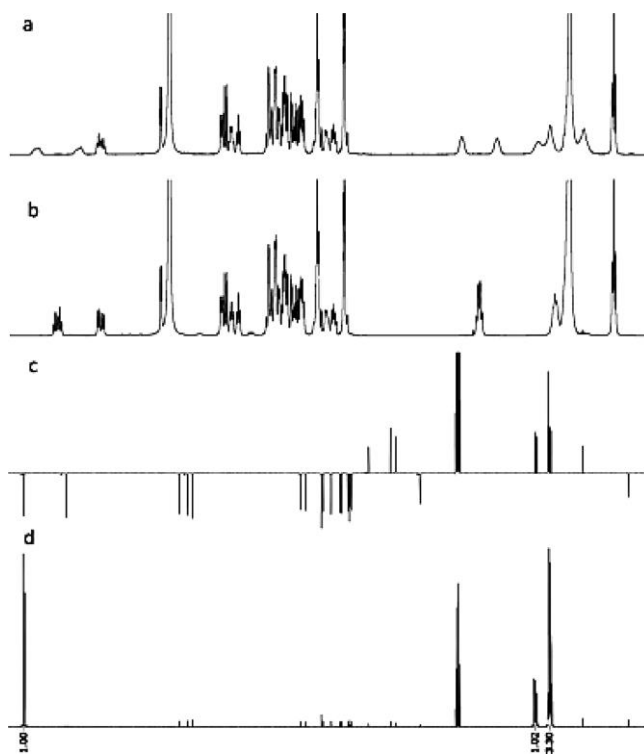


Figure 2.2 ^1H - and ^{13}C NMR spectra of globotriaosylsphingosine both in ^{13}C -enriched (**41b**) and unenriched (**41a**) form. (a) 400 MHz ^1H NMR spectrum ($[\text{D}_4]$ methanol) of **41b**, in which the ^{13}C , ^1H coupling of the double-bond proton is apparent. (b) 400 MHz ^{13}C -decoupled ^1H NMR spectrum ($[\text{D}_4]$ methanol) of **41b**. (c) 151.1 MHz ^{13}C NMR spectrum ($[\text{D}_4]$ methanol) of **41a**. (d) 151.1 MHz ^{13}C NMR spectrum ($[\text{D}_4]$ methanol) of **41b**, with integration of the ^{13}C labels.

2.4 Conclusion

In conclusion, a comprehensive library of stable-isotope-enriched sphingolipids has been constructed by straightforward synthetic routes taking into consideration that the synthesis of ^{13}C -enriched lipids with the carbons introduced at specific predetermined sites can be executed with only a limited number of reagents available from commercial sources. The key step in the assembly of the sphingosine backbone, the cross-metathesis reaction between the sphingosine head-group alkene and the long-chain alkene, was optimized to minimize truncation of the long-chain alkene before the cross-metathesis event. Elimination of one or two methylene units, leading to the loss of ^{13}C -labels, was observed during this reaction under conditions previously described. The addition of acetic acid to the reaction mixture effectively prevented the truncation of the alkene chain. With this work we believe we have obtained a valuable set of molecular probes to study sphingolipid metabolism in healthy and disease states in a chemical metabolomics

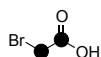
setting. The route is also flexible, and is thus amenable for the production of other sphingolipid metabolites, with respect to both the polar head group, such as for instance phosphate and phosphate diesters, and also the *N*-acyl-substituted fatty acid moiety.

2.3 Experimental section

General Remarks: [$^{13}\text{C}_2$]-acetic acid (99.95% isotopically pure, product code CLM-105), potassium [^{13}C]-cyanide (99% isotopically pure, product code CLM-297), and [1,2,3- $^{13}\text{C}_3$]-myristic acid (99% isotopically pure, product code CLM-3665) were purchased from Cambridge Isotope Laboratories, Inc., and were used as received. Commercially available reagents and solvents (Acros, Fluka, or Merck) were used as received, unless otherwise stated. CH_2Cl_2 and THF were freshly distilled before use, over P_2O_5 and Na/benzophenone, respectively. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide. Traces of water were removed from starting compounds by coevaporation with toluene. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out using forced flow of the indicated solvent systems on Screening Devices silica gel 60 (40–63 μm mesh). Size-exclusion chromatography was carried out on Sephadex LH20 ($\text{MeOH}/\text{CH}_2\text{Cl}_2$, 1:1). Analytical TLC was carried out on aluminium sheets (Merck, silica gel 60, F254). Compounds were visualized by UV absorption (254 nm), or by spraying with ammonium molybdate/cerium sulphate solution [$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4 \text{H}_2\text{O}$ (25 g/L), $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_6 \cdot 2 \text{H}_2\text{O}$ (10 g/L), 10 % sulphuric acid in ethanol] or phosphomolybdic acid in EtOH (150 g/L), followed by charring (ca. 150 °C). IR spectra were recorded with a Shimadzu FTIR-8300 instrument and are reported in cm^{-1} . Optical rotations were measured with a Propol automatic polarimeter (sodium D-line, $\lambda = 589 \text{ nm}$). ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 MHz spectrometer at 400.2 (^1H) and 100.6 (^{13}C) MHz, or with a Bruker AV 600 MHz spectrometer at 600.0 (^1H) and 151.1 (^{13}C) MHz. Chemical shifts are reported as δ values (ppm), and were referenced to tetramethylsilane ($\delta = 0.00 \text{ ppm}$) directly in CDCl_3 , or using the residual solvent peak (D_2O). Coupling constants (*J*) are given in Hz, and all ^{13}C spectra were proton decoupled. NMR assignments were made using COSY and HSQC, and in some cases TOCSY experiments. LC–MS analysis was carried out with an LCQ Advantage Max (Thermo Finnigan) instrument equipped with a Gemini C18 column (Phenomenex, 50 \times 4.6 mm, 3 μm), using the following buffers: A: H_2O , B: acetonitrile, and C: aq. TFA (1.0 %). HPLC–MS purifications were carried out with an Agilent Technologies 1200 Series automated HPLC system with a Quadrupole MS 6130, equipped with a semi-preparative Gemini C18 column (Phenomenex, 250 \times 10.00, 5 μm). Products were eluted using the following buffers: A: aq. TFA (0.2 %), B: acetonitrile (HPLC-grade), 5 mL/min. Purified products were lyophilized with a CHRIST ALPHA 2–4 LDPLUS apparatus to remove water and traces of buffer salts.

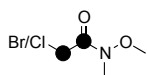
General procedure for the synthesis of ceramides from the sphingosines. Sphingosine (0.1 mmol) was dissolved in THF (12 mL) and sat. aq. NaOAc (10 mL) was added. Palmitoyl chloride (0.13 mmol, 1.3 eq) was added and the reaction was stirred vigorously at room temperature for 3 hours. The mixture was diluted with THF (20 mL) and washed with water (10 mL). The water layer was extracted with THF (3 \times 20 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The ceramides were purified by column chromatography (chloroform/MeOH) and HPLC–MS, using a C₄ column. Products were eluted using the following buffers: A: 25 nM NH_4OAc in $\text{MeOH}/\text{H}_2\text{O}$ (3:1), B: acetonitrile (HPLC-grade). Purified products were lyophilized to remove water and traces of buffer salts. The symbol * in the NMR analysis stands for the palmitate group of the ceramide.

[$^{13}\text{C}_2$]-2-Bromoacetic acid (10). Trifluoroacetic anhydride (67.3 mL, 484 mmol, 3.0 eq) was slowly added to [1,2- $^{13}\text{C}_2$]-acetic acid **9** (10 g, 161 mmol, 1.0 eq), under stirring. Bromine (8.30 mL, 161 mmol, 1.0 eq) was added and the reaction was stirred at room temperature for 20 h. The reaction mixture was cooled to 0 °C followed by addition of water (10.2 mL, 564 mmol, 3.5 eq). Excess bromine was removed by a flow of argon. The crude mixture was then dissolved in toluene (200 mL) and concentrated



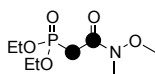
in vacuo. This procedure was repeated twice giving [$^{13}\text{C}_2$]-2-bromoacetic acid **10** as an off-white solid without further purification (23.2 g, 142 mmol, 88%). Analytical data are in agreement with the literature.^[8]

[1,2- $^{13}\text{C}_2$]-2-Bromo-*N*-methoxy-*N*-methylacetamide and [1,2- $^{13}\text{C}_2$]-2-Chloro-*N*-methoxy-*N*-methylacetamide



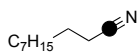
(11). [$^{13}\text{C}_2$]-2-Bromoacetic acid **10** (8.46 g, 60 mmol, 1.0 eq) was then dissolved in anhydrous DCM (100 mL), put under an atmosphere of argon, and cooled to 0 °C. Oxalyl chloride (10.5 mL, 120 mmol, 2.0 eq) was added followed by a drop of DMF. The reaction was then kept under a flow of argon and continuous stirring at room temperature. When gas evolution stopped (~ 2 h), 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 (12.3 mL, 168 mmol, 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 2 h. The reaction mixture was then stirred at room temperature for 30 min. The solids were filtered over a Whatmann paper and washed with DCM. The eluent was concentrated *in vacuo* and purified by column chromatography (10–40% EtOAc in petroleum ether), giving [1,2- $^{13}\text{C}_2$]-2-Bromo-*N*-methoxy-*N*-methylacetamide and [1,2- $^{13}\text{C}_2$]-2-Chloro-*N*-methoxy-*N*-methylacetamide in a 4:1 ratio (as determined by ^1H - and ^{13}C -NMR) as a clear oil (10.25 g, 58.3 mmol, 97%). R_f = 0.35 (30% EtOAc in petroleum ether); [1,2- $^{13}\text{C}_2$]-2-Bromo-*N*-methoxy-*N*-methylacetamide: ^1H NMR (400 MHz, CDCl_3) δ 4.01 (dd, 2 H, J = 154.0, 3.6 Hz, H-2), 3.80 (s, 3 H, $\text{CH}_3\text{-OMe}$), 3.24 (s, 3 H, $\text{CH}_3\text{-NMe}$); ^{13}C NMR (101 MHz, CDCl_3) δ 167.5 (d, J = 58.5 Hz, C=O), 61.6 ($\text{CH}_3\text{-OMe}$), 32.5 ($\text{CH}_3\text{-NMe}$), 25.1 (d, J = 58.4 Hz, CH_2); HRMS calculated for [$\text{C}_2^{13}\text{C}_2\text{H}_8\text{NO}_2\text{Br} + \text{H}$] $^+$: 183.9878, found 183.9877. [1,2- $^{13}\text{C}_2$]-2-Chloro-*N*-methoxy-*N*-methylacetamide: ^1H NMR (400 MHz, CDCl_3) δ 4.25 (dd, 2 H, J = 152.3, 4.4 Hz, H-2), 3.76 (s, 3 H, $\text{CH}_3\text{-OMe}$), 3.24 (s, 3 H, $\text{CH}_3\text{-NMe}$); ^{13}C NMR (101 MHz, CDCl_3) δ 167.5 (d, J = 57.2 Hz, C=O), 61.6 ($\text{CH}_3\text{-OMe}$), 40.7 (d, J = 57.7 Hz, CH_2), 32.5 ($\text{CH}_3\text{-NMe}$); HRMS calculated for [$\text{C}_2^{13}\text{C}_2\text{H}_8\text{NO}_2\text{Cl} + \text{H}$] $^+$: 140.0383 found 140.0381.

Diethyl-([1,2- $^{13}\text{C}_2$]-*N*-methoxy-*N*-methylcarbamoylmethyl) phosphonate (12).



methoxy-*N*-methylacetamide **11** (10.25 g, 58.3 mmol, 1.0 eq) and triethylphosphite (10.5 mL, 60 mmol, 1.05 eq) were put in a round bottom flask equipped with an 15 cm air cooled condenser and heated for 3 h at 150 °C. The crude mixture was cooled down and directly purified by column chromatography (30–50% acetone in petroleum ether), giving the title compound **12** as a clear oil (13.7 g, 56.8 mmol, 95%). R_f = 0.20 (40% acetone in petroleum ether); ^1H NMR (400 MHz, CDCl_3) δ 4.24 – 4.13 (m, 4 H, $\text{CH}_2\text{-OEt}$ x2), 3.79 (s, 3 H, $\text{CH}_3\text{-OMe}$), 3.22 (s, 3 H, $\text{CH}_3\text{-NMe}$), 3.16 (ddd, 2 H, J = 129.8, 21.9, 6.6 Hz, H-2), 1.35 (t, 6 H, J = 7.1 Hz, $\text{CH}_3\text{-OEt}$ x 2); ^{13}C NMR (101 MHz, CDCl_3) δ 165.5 (dd, J = 53.1, 4.5 Hz, C=O), 62.0, 61.9 ($\text{CH}_2\text{-OEt}$ x 2), 60.9 ($\text{CH}_3\text{-OMe}$), 31.57 ($\text{CH}_3\text{-NMe}$), 30.9 (dd, J = 136.1, 53.1 Hz, H-2), 15.82, 15.76 ($\text{CH}_3\text{-OEt}$ x2); IR (neat): 2984, 1658, 1423, 1381, 1253, 1018, 961, 789 cm^{-1} ; HRMS calculated for [$\text{C}_6^{13}\text{C}_2\text{H}_{18}\text{NO}_5\text{P} + \text{H}$] $^+$: 242.1063, found 242.1064.

[1- $^{13}\text{C}_1$]-Decanitrile (14).



$^{13}\text{C}_1$ -Potassium cyanide (5.00 g, 76.0 mmol, 1.0 eq) was added to a solution of 1-bromononane **13** (16.5 g, 79.0 mmol, 1.05 eq) in a mixture of ethanol/water (9:1, 140 mL) and heated over night at 80 °C. 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); ^1H 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, 3 H, 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 calculated for [$\text{C}_9^{13}\text{CH}_{19}\text{N} + \text{H}$] $^+$: 155.2623, found 155.2624.

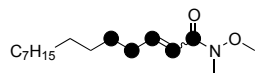
[1-¹³C₁]-Decanal (15). [1-¹³C₁]-Decanitrile **14** (11.1 g, 72.0 mmol, 1.0 eq) was dissolved in anhydrous THF (250 mL) and cooled to 0 °C before addition of DIBAL-H (1.5 M in hexanes, 52.9 mL, 79.0 mmol, 1.1 eq). The reaction mixture was stirred at room temperature for 2.5 h. The mixture was then transferred to an extraction funnel, diluted with ether (200 mL), and washed with 1 M HCl (2 x 400 mL), sat. aq. NaHCO₃ (400 mL). The water layers were extracted with ether (2 x 400 mL) and the combined organics were dried (MgSO₄), 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, CDCl₃) 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, 3 H, *J* = 6.9 Hz); ¹³C NMR (101 MHz, CDCl₃) δ 203.0 (C=O), 43.9 (d, *J* = 38.8, C-2), 31.8, 29.35, 29.32, 29.2 (CH₂ x4), 29.1 (d, *J* = 3.4 Hz, C-4), 22.6 (CH₂), 22.0 (d, *J* = 1.6 Hz, C-3), 14.0 (C-10); IR (neat): 2922, 2855, 1728, 1466, 719 cm⁻¹.

[1,2,3-¹³C₃]-(*E/Z*)-*N*-Methoxy-*N*-methyl-dodec-2-en-amide (16). Diethyl-([1,2-¹³C₂]-*N*-methoxy-*N*-methylcarbamoylmethyl)phosphonate **12** (10.4 g, 43.1 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, 42.3 mmol, 1.08 eq). The reaction mixture was stirred for 10 min at 0 °C. [1-¹³C₁]-Decanal **15** (6.16 g, 39.2 mmol, 1.0 eq) dissolved in anhydrous THF (40 mL) was added to the phosphonate carbanion and the reaction mixture 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 (Na₂SO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (0–15% EtOAc in petroleum ether), giving [1,2,3-¹³C₃]-(*E*)-*N*-Methoxy-*N*-methyl-dodec-2-en-amide (7.52 g, 30.8 mmol, 79%) and [1,2,3-¹³C₃]-(*Z*)-*N*-Methoxy-*N*-methyl-dodec-2-en-amide (0.75 mg, 3.07 mmol, 8%) in a combined yield of 87% as clear oil. *R*_f **16E** = 0.42; **16Z** = 0.64 (20% EtOAc in petroleum ether); (*E*-isomer, **16E**) ¹H NMR (400 MHz, CDCl₃) 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, CH₃-OMe), 3.24 (s, 3 H, CH₃-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); ¹³C NMR (101 MHz, CDCl₃) 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 (CH₃-OMe), 32.5 (m, C-4), 32.3 (m, CH₃-NMe), 31.9, 29.5, 29.4, 29.3 (CH₂ x4), 29.2 (d, *J* = 3.6 Hz, C-6), 28.3 (m, C-5), 22.7 (CH₂), 14.1 (C-12); IR (neat): 2926, 5856, 1622, 1584, 1462, 1368, 1175, 993 cm⁻¹; HRMS calculated for [C₁₁¹³C₃H₂₇NO₂H]⁺: 245.2215, found 245.2216; (*Z*-isomer, **16Z**) ¹H NMR (400 MHz, CDCl₃) 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, CH₃-OMe), 3.21 (s, 3 H, CH₃-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, 3 H, *J* = 6.9 Hz, H-12); ¹³C NMR (101 MHz, CDCl₃) δ 167.6 (d, *J* = 63.6, C=O), 147.8 (d, *J* = 67.1 Hz, C-3), 117.9 (dd, *J* = 67.1, 63.6 Hz, C-2), 61.5 (CH₃-OMe), 31.9, 31.6 (CH₃-NMe), 29.6, 29.5 (CH₂ x3), 29.38 (d, *J* = 4.0 Hz, C-6), 29.35 – 29.29 (m, CH₂ x2), 29.1 (m, C-3), 22.7 (CH₂), 14.1 (C-12); IR (neat): 2925, 2855, 1618, 1459, 1334, 1178, 996, 776 cm⁻¹; HRMS calculated for [C₁₁¹³C₃H₂₇NO₂ + H]⁺: 245.2215, found 245.2216.

[1,2,3-¹³C₃]-*N*-methoxy-*N*-methyl-dodecanamide (17). [1,2,3-¹³C₃]-(*E/Z*)-*N*-Methoxy-*N*-methyl-dodec-2-en-amide **16E** and **16Z** (8.25 g, 33.8 mmol, 1.0 eq) was dissolved in EtOAc (200 mL). The solution was bubbled 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 min and left over night under a hydrogen atmosphere. 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 [1,2,3-¹³C₃]-*N*-methoxy-*N*-methyl-dodecanamide as a clear oil (6.85 g, 27.8 mmol, 82%). *R*_f = 0.38 (20% EtOAc in petroleum ether); ¹H NMR (400 MHz, CDCl₃) δ 3.68 (s, 3 H, CH₃-OMe), 3.18 (s, 3 H, CH₃-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); ¹³C NMR (101 MHz, CDCl₃) δ 174.6 (bd, *J* = 51.5 Hz, C=O), 61.1 (CH₃-OMe), 31.9 (CH₃-NMe), 31.8 (dd, *J* = 51.5, 37.5 Hz, C-2), 29.7 – 29.1 (m, CH₂ x7), 24.6

(dd, $J = 34.9, 1.3$ Hz, C-3), 22.6 (CH₂), 14.1 (C-12); IR (neat): 2923, 2854, 1627, 1464, 1369, 1174, 1119, 998, 722, 436 cm⁻¹; HRMS calculated for [C₁₁¹³C₃H₂₉NO₂ + H]⁺: 247.2372, found 247.2373.

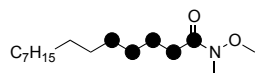
[1,2,3,4,5-¹³C₅]-(*E/Z*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (18). [1,2,3-¹³C₃]-*N*-Methoxy-*N*-methyl-



dodecanamide **17** (3.91 g, 15.9 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) (2.38 mL, 9.52 mmol, 0.6 eq). The reaction mixture was stirred for 45 min and was then cooled to -15 °C, before addition of sat. aq. KHSO₄ (100 mL) and diethylether (300 mL). The two-phase system was stirred vigorously for 30 min and was then dried with MgSO₄ followed by Na₂SO₄. The solids were filtered and washed with diethylether (200 mL). The eluate was concentrated *in vacuo*, giving crude [1,2,3-¹³C₃]-dodecanal (2.96 g, 15.8 mmol) as a clear oil which was used without further purification.

Diethyl (*N*-Methoxy-*N*-methyl-carbamoylmethyl)phosphonate **12** (4.20 g, 17.4 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 [1,2,3-¹³C₃]-dodecanal was dissolved in anhydrous THF (20 mL) and added to the Horner-Wadsworth-Emmons reagent at 0 °C. The reaction mixture was then stirred at room temperature 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 (Na₂SO₄), filtered, and concentrated *in vacuo*. Purification by column chromatography (5–15% EtOAc in petroleum ether) giving [1,2,3,4,5-¹³C₅]-(*E*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (3.05 g, 11.1 mmol, 70%) and [1,2,3,4,5-¹³C₅]-(*Z*)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (310 mg, 1.13 mmol, 7%) in a combined yield of 77% as clear oils. R_f **18E** = 0.39; **18Z** = 0.58 (15% EtOAc in petroleum ether). (*E*-isomer, **18E**) ¹H NMR (600 MHz, CDCl₃) 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, CH₃-OMe), 3.24 (s, 3 H, CH₃-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); ¹³C NMR (151 MHz, CDCl₃) 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 (CH₃-OMe), 32.5 (dddd, $J = 41.8, 33.7, 6.1, 1.5$ Hz, C-4), 32.3 (CH₃-NMe), 31.9 (CH₂), 29.6 – 29.0 (m, CH₂ x6), 28.3 (ddd, $J = 33.7, 3.6, 2.1$ Hz, C-5), 22.7 (CH₂), 14.1 (C-12); IR (neat): 2924, 2854, 1618, 1583, 1464, 1368, 991 cm⁻¹; HRMS Calculated for [C₁₁¹³C₅H₃₁NO₂ + H]⁺: 275.2595, found 275.2595; (*Z*-isomer, **18Z**) ¹H NMR (600 MHz, CDCl₃) 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, CH₃-OMe), 3.21 (s, 3 H, CH₃-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); ¹³C NMR (151 MHz, CDCl₃) 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 (CH₃-OMe), 32.0 (CH₃-NMe), 31.9 (CH₂), 30.2 – 28.4 (m, CH₂ x8), 22.7 (CH₂), 14.1 (C-12); IR (neat): 2923, 2854, 1618, 1464, 1331, 1176, 1086, 999, 775 cm⁻¹; HRMS calculated for [C₁₁¹³C₅H₃₁NO₂ + H]⁺: 275.2595, found 275.2595.

[1,2,3,4,5-¹³C₅]-*N*-methoxy-*N*-methyl-tetradecanamide (19). [1,2,3,4,5-¹³C₅]-(*E/Z*)-*N*-Methoxy-*N*-methyl-



tetradec-2-enamide **18E/Z** (3.20 g, 11.66 mmol, 1.0 eq) was dissolved in EtOAc (100 mL). The solution was bubbled 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 min and was then left over night under a hydrogen atmosphere. 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) yielded [1,2,3,4,5-¹³C₅]-*N*-methoxy-*N*-methyl-tetradecanamide as a clear oil (3.00 g, 10.85 mmol, 93%). R_f = 0.38 (15% EtOAc in petroleum ether); ¹H NMR (600 MHz, CDCl₃) δ 3.68 (s, 3 H, CH₃-OMe), 3.18 (s, 3 H, CH₃-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); ¹³C NMR (151 MHz, CDCl₃) δ 174.8 (dm, $J = 51.5$ Hz, C=O), 61.1 (CH₃-OMe), 32.1 (CH₃-NMe), 31.9 (dd, $J = 51.5, 35.6$ Hz, C-2), 29.7 – 29.1 (m, CH₂ x9), 24.6 (m, C-3), 22.6 (CH₂), 14.1 (C-14); IR (neat): 2922, 2853, 1628, 1458, 1370, 1175, 996, 721 cm⁻¹; HRMS calculated for [C₁₁¹³C₅H₃₃NO₂ + H]⁺: 277.2751, found 277.2752.

[2,3,4,5,6-¹³C₅]-Pentadec-1-ene (20). [1,2,3,4,5-¹³C₅]-*N*-Methoxy-*N*-methyl-tetradecanamide **19** (1.57 g, 5.72 mmol, 1.0 eq) was dissolved in anhydrous THF (55 mL) and LiAlH₄ (4 M in THF) (0.86 mL, 3.43 mmol, 0.6 eq) was added at 0 °C. The reaction mixture was stirred for 45 minutes and then cooled to ca -15 °C before addition of sat. aq. KHSO₄ (40 mL) and diethylether (100 mL). The resulting two phase mixture was stirred vigorously for 30 min and then dried with MgSO₄, and then Na₂SO₄. The solids were filtered and washed with diethylether (100 mL). The eluate was concentrated *in vacuo* giving crude [1,2,3,4,5-¹³C₅]-tetradecanal (1.24 g, 5.72 mmol) as a clear oil which was used without further purification.

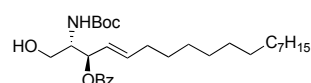
Methyltriphenylphosphonium bromide (3.06 g, 8.58 mmol, 1.5 eq) was suspended in anhydrous THF (150 mL) and *n*-butyllithium (1.6 M in hexanes) (4.65 mL, 7.44 mmol, 1.3 eq) was added at 0 °C. The reaction was then stirred for 10 min at 0 °C. The crude [1,2,3,4,5-¹³C₅]-tetradecanal was dissolved in 20 mL anhydrous THF and then added to the phosphorylide at 0 °C. The reaction mixture was stirred over night at room temperature and transferred to an extraction funnel using ether (100 mL). The reaction mixture was washed with water (200 mL x 2) and brine (200 mL). The water phases were extracted with ether (200 mL) and the combined organics were dried (Na₂SO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (100% petroleum ether) produced the title compound **20** as a clear oil (1.15 g, 5.34 mmol, 93%). *R*_f = 0.98 (100% petroleum ether); ¹H NMR (400 MHz, CDCl₃) 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_ε), 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); ¹³C NMR (101 MHz, CDCl₃) 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 (CH₂), 29.9 – 28.6 (m, CH₂ x9), 22.7 (CH₂), 14.1 (C-15); IR (neat): 2922, 2853, 1628, 1458, 1370, 1175, 1117, 996, 721 cm⁻¹.

(E)-1,2-*O*,*N*-Isopropyliden-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine (22a). (2*S*,3*R*)-2-Amino-*N*-(*tert*-butoxycarbonyl)-1,3-dihydroxy-1,2-*O*,*N*-isopropylidene-4-pentene **21** (1 g, 4.0 mmol, 1.0 eq) and pentadec-1-ene (1.70 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) and acetic acid (45 μL, 0.79 mmol, 0.2 eq). The reaction was refluxed under a flow of argon for 36 h. The reaction 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 (1.30 g, 2.96 mmol, 74%). *R*_f = 0.19 (10% EtOAc in petroleum ether); [α]_D²²: -26 (c = 0.25 CHCl₃); ¹H 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, CH₃-acetonide), 1.43 (m, 12 H, CH₃-acetonide and CH₃-*t*Bu-Boc), 1.39–1.20 (m, 22 H, H-7 to H-17), 0.87 (t, 3 H, *J* = 6.6 Hz, H-18); ¹³C 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-acetonide), 78.7 (C_q-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, CH₃-*t*Bu-Boc and CH₃-acetonide x2), 13.2 (C-18). IR (neat): 3436, 2924, 2854, 1702, 1381, 1365, 1255, 1173, 1097, 848, 766 cm⁻¹; HRMS calculated for [C₂₆H₄₉NO₄ + H]⁺: 440.3734, found 440.3733.

(E)-[5,6,7,8,9-¹³C₅]-1,2-*O*,*N*-Isopropylidene-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine (22b) (2*S*,3*R*)-2-Amino-*N*-(*tert*-butoxycarbonyl)-1,3-dihydroxy-1,2-*O*,*N*-isopropylidene-4-pentene **21** (3.58 g, 13.9 mmol, 3.0 eq) and [2,3,4,5,6-¹³C₅]-pentadec-1-ene **20** (1.00 g, 4.64 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) and acetic acid (53 μL, 0.93 mmol, 0.2 eq). The reaction was refluxed under a flow of argon for 36 h. The reaction 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 (1.68 g, 3.29 mmol, 81%). *R*_f = 0.19 (10% EtOAc in petroleum ether); [α]_D²²: -19 (c = 0.5 CHCl₃); ¹H 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-

1a), 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, $J = 124.2$ Hz, H-6), 1.56 – 1.06 (m, 37 H, CH₃-*t*Bu-Boc, CH₃-acetonide and H-7 to H-17), 0.87 (t, 3 H, $J = 6.9$ Hz, H-18); ¹H NMR (400 MHz, CDCl₃) 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, CH₃-*t*Bu-Boc, CH₃-acetonide and H-7 to H-17), 0.88 (t, 3 H, $J = 6.8$ Hz, H-18); ¹H NMR (400 MHz, CDCl₃, ¹³C-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, CH₃-*t*Bu-Boc, CH₃-acetonide and H-7 to H-17), 0.88 (t, 3 H, $J = 6.8$ Hz, H-18); ¹³C 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-acetonide), 78.4 (C_q-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-6_{sp} and CH₂-_{sp}), 29.7 – 26.1 (CH₂-_{sp} x10, CH₃-*t*Bu-Boc and CH₃-acetonide x2), 21.5 (CH₂-_{sp}), 13.2 (C-18_{sp}); IR (neat): 3436, 2922, 2853, 1698, 1458, 1386, 1365, 1256, 1173, 1098, 965, 848, 766 cm⁻¹; HRMS calculated for [C₂₁¹³C₅H₄₉NO₄ + H]⁺: 445.3902, found 445.3902.

3-O-Benzoyl-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine (23a).

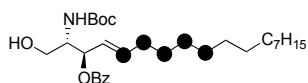


(*E*)-1,2-*O*-*N*-Isopropylidene-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine **22a** (0.59 g, 1.3 mmol, 1.0 eq) was dissolved in a mixture of 2:1 pyridine and DCM (10 mL). DMAP (16 mg, 0.13 mmol, 0.1 eq) was added followed by benzoyl chloride (0.23 mL, 2.0 mmol, 1.5 eq). The reaction was stirred 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. NaHCO₃ (50 mL) and brine (50 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organic layers were dried (Na₂SO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (1.5% EtOAc in petroleum ether) 1,2-*O*-*N*-Isopropylidene-3-*O*-benzoyl-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine as a clear oil. (0.61 g, 1.1 mmol, 84%). $R_f = 0.82$ (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: -29 ($c = 0.66$ CHCl₃); ¹H 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, CH₃-*t*Bu-Boc), 1.40 (s, 3 H, CH₃-acetonide), 1.36-1.17 (m, 25 H, CH₃-acetonide and H-7 to H-17), 0.86 (t, 3 H, H-18); ¹³C 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-arom), 128.9, 128.1 (CH_{arom} x2), 125.4 (C-4), 93.2 (C_q-acetonide), 79.1 (C_q-Boc), 73.4 (C-3), 62.9 (C-1), 59.1 (C-2), 31.1, 30.8, 28.5 (x2), 28.4 (x2), 28.4, 28.2, 27.8 (x2), 27.6 (x2), 21.5 (C-7 to C-17, CH₃-*t*Bu-Boc and CH₃-acetonide x2), 13.3 (C-18); IR (neat): 2924, 2854, 1724, 1701, 1365, 1268, 1097, 1070, 855, 709 cm⁻¹; HRMS calculated for [C₃₃H₅₃NO₅ + Na]⁺: 566.3816, found 566.3814.

1,2-*O*-*N*-Isopropylidene-3-*O*-benzoyl-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine (0.5 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 room temperature over night and was the 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. NaHCO₃ (60 mL) and brine (50 mL). The water layers were back extracted with EtOAc (60 mL). The combined organics were dried (Na₂SO₄), 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 recovering starting material). $R_f = 0.07$ (10% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +15 ($c = 1.0$ CHCl₃); ¹H NMR (400 MHz, CDCl₃) 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, CH₃-*t*Bu-Boc), 1.40-1.20 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, $J = 6.8$ Hz, H-18); ¹³C NMR (100 MHz, CDCl₃) δ 166.2 (C=O_{Bz}), 155.8 (C=O_{Boc}), 137.3 (C-5), 133.2 (CH_{arom}), 129.8 (C_q-arom), 129.7, 128.4 (CH_{arom} x2), 124.6 (C-4), 79.6 (C_q-Boc), 74.8 (C-3), 61.7 (C-1), 54.5 (C-2), 32.2 (C-6), 31.9, 29.62 (x3), 29.60, 29.5, 29.4, 29.3, 29.2, 28.9, (C_{sp} x10), 28.3 (CH₃-*t*Bu-Boc), 22.6 (C_{sp}), 14.1 (C-18); IR (neat): 3372, 2924, 2854, 1715, 1268, 1171, 1111, 1070, 969, 710 cm⁻¹; HRMS calculated for [C₃₀H₄₉NO₅ + Na]⁺: 526.3503, found 526.3500.

[5,6,7,8,9-¹³C₅]-3-O-Benzoyl-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine (23b). [5,6,7,8,9-¹³C₅]-1,2-*O*-*N*-

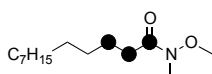


Isopropylidene-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine **22b** (1.14 g, 2.56 mmol, 1.0 eq) was dissolved in a 2:1 mixture of pyridine and DCM (20 mL). DMAP (16 mg, 0.13 mmol, 0.05 eq) was added followed by benzoyl chloride (0.45 mL, 3.85 mmol, 1.5 eq). The reaction mixture was

stirred over night and was then quenched with methanol (0.5 mL). The reaction solvent was removed *in vacuo* and the resulting residue was dissolved in EtOAc (50 mL), washed with 1M HCl (50 mL), sat. aq. NaHCO₃ (50 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were dried (Na₂SO₄), filtered, and concentrated *in vacuo*. Purification by column chromatography (1.5% EtOAc in petroleum ether) giving [5,6,7,8,9-¹³C₅]-1,2-*O*,*N*-isopropylidene-3-*O*-benzoyl-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine as a clear oil (1.13 g, 2.37 mmol, 92%). *R*_f = 0.29 (5% EtOAc in petroleum ether); [α]_D²²: -30 (c = 0.5 CHCl₃); ¹H 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, CH₃-*t*Bu-Boc, CH₃-acetonide x2 and H-7 to H-17), 0.86 (t, 3 H, *J* = 6.3 Hz, H-18); ¹³C NMR (101 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-arom}), 128.9, 128.2 (CH_{arom} x2), 125.2 (d, *J* = 72.2 Hz, C-4), 93.2 (C_{q-acetonide}), 79.2 (C_{q-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 CH₂), 28.8 – 27.3 (m, CH₂ x9, CH₃-*t*Bu-Boc and CH₃-acetonide x2), 21.6 (CH₂), 13.4 (C-18); The same sample in CDCl₃ at room temperature shows two rotamers: ¹H NMR (400 MHz, CDCl₃) 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, CH₃-*t*Bu-Boc, CH₃-acetonide x2 and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.9 Hz, H-18); ¹³C NMR (100 MHz, CDCl₃) 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-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-acetonide} x2), 80.4, 80.2 (C_{q-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 CH₂), 29.8 – 28.2 (m, CH₂ x9, CH₃-*t*Bu-Boc and CH₃-acetonide x2), 22.7 (CH₂), 14.1 (C-18); HRMS calculated for [C₂₈¹³C₅H₅₃NO₅ + Na]⁺: 571.3984, found 571.3982.

[5,6,7,8,9-¹³C₅]-1,2-*O*,*N*-isopropylidene-3-*O*-benzoyl-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine (120 mg, 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 mixture was stirred over night at room temperature. The reaction mixture was transferred to an extraction funnel using EtOAc (60 mL) and washed with sat. aq. NaHCO₃:water 2:1 (60 mL) and brine (50 mL). The water layer was extracted with EtOAc (60 mL). The combined organics were dried (Na₂SO₄), filtered, and concentrated *in vacuo*. Purification by column chromatography (5-10% EtOAc in petroleum ether) produced the title compound **23b** as an amorphous solid (70 mg, 0.14 mmol, 63%; 83% based on recovered starting material). *R*_f = 0.07 (10% EtOAc in petroleum ether); [α]_D²²: +16 (c = 0.5 CHCl₃); ¹H NMR (400 MHz, CDCl₃) 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, CH₃-*t*Bu-Boc and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ¹³C NMR (101 MHz, CDCl₃) 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-arom}), 129.75, 128.4 (CH_{arom} x2), 124.6 (d, *J* = 71.5 Hz, C-4), 79.7 (C_{q-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 CH₂), 29.8 – 28.1 (CH₂ x9 and CH₃-*t*Bu-Boc), 22.7 (CH₂), 14.1 (C-18); IR (neat): 3372, 2922, 2853, 1696, 1505, 1452, 1267, 1169, 1111, 1070, 1026, 966, 710 cm⁻¹; HRMS calculated for [C₂₅¹³C₅H₄₉NO₅ + Na]⁺: 531.3671, found 531.3667.

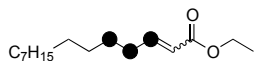
[1,2,3-¹³C₃]-*N*-Methoxy-*N*-(methyl)-tetradecanamide (25). [1,2,3-¹³C₃]-myristic acid **24** (3.00 g, 13.0 mmol, 1.0



eq) was dissolved in anhydrous DCM (26 mL), put under an atmosphere of argon and cooled to 0 °C. Oxalyl chloride (2.28 mL, 26.0 mmol, 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 (~ 2 h), the reaction was concentrated *in vacuo*. The residue was

dissolved in anhydrous DCM (13 mL) and cooled to -78°C . *N,O*-Dimethylhydroxylamine (2.30 mL, 32.5 mmol, 2.5 eq), dissolved in anhydrous DCM (13 mL), was slowly added to the myristoyl chloride at -78°C . Then the reaction was left stirring, reaching room temperature over 2 h. The reaction was stirred at room temperature for 30 min. The solids were filtered over a Whatmann paper and washed with DCM. The mother liquor was concentrated *in vacuo* and purified by column chromatography (5–20% EtOAc in Pentane), giving the title product as a clear oil (3.45 g, 12.7 mmol, 98%). $R_f = 0.42$ (20% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 3.68 (s, 3 H, $\text{CH}_3\text{-OMe}$), 3.13 (d, $J = 2.0$ Hz, 3 H, $\text{CH}_3\text{-NMe}$), 2.41 (dm, 2 H, $J = 127.2$ Hz, H-2), 1.62 (dm, 2 H, $J = 128.8$ Hz, H-3), 1.35–1.22 (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 175.0 (d, $J = 51.0$ Hz, C=O), 61.4 ($\text{CH}_3\text{-OMe}$), 32.04 ($\text{CH}_3\text{-NMe}$), 31.99 (dd, $J = 51.0, 34.0$ Hz, C-2), 29.8–29.3 (m, $\text{CH}_2 \times 9$), 24.77 (dd, $J = 35.0, 2.0$ Hz, C-3), 22.8 (CH_2), 14.2 (C-16); IR (neat): 2924, 2855, 1616, 1462, 1375, 1176, 908, 729 cm^{-1} ; HRMS calculated for $[\text{C}_{13}\text{-}^{13}\text{C}_3\text{H}_{33}\text{NO}_2 + \text{H}]^+$: 275.2612, found 275.2683.

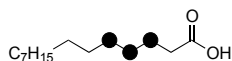
Ethyl (E)-[3,4,5- $^{13}\text{C}_3$]-hexadec-2-enoate (27). [1,2,3- $^{13}\text{C}_3$]-*N*-(Methoxy)-*N*-Methyl-tetradecanamide **25** (2.74 g, 10.0 mmol, 1.0 eq) was dissolved in dry THF (20 mL) and cooled to -78°C , before addition of DIBAL-H (1.5 M in toluene) (8.0 mL, 12.0 mmol, 1.2 eq). The reaction was stirred for 30 min before being quenched with sat. aq. Rochelle salt (12 mL).



The mixture was then transferred to an extraction funnel with EtOAc (50 mL) and washed with water (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (50 mL). The combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo* giving crude [1,2,3- $^{13}\text{C}_3$]-tetradecanal (2.15 g, 10.0 mmol) as a clear oil which was used without further purification. Triethyl phosphonoacetate **26** (3.14 g, 14.0 mmol, 1.4 eq) was dissolved in dry THF (50 mL) and cooled to 0°C before addition of *n*-butyllithium (1.6 M in hexanes, 7.8 mL, 12.5 eq). The reaction was stirred for 10 min at 0°C . The crude [1,2,3- $^{13}\text{C}_3$]-tetradecanal was dissolved in anhydrous THF (10 mL) and added to the Horner-Wadsworth-Emmons reagent at 0°C . The mixture was then stirred over night at room temperature. The mixture was transferred to an extraction funnel with ether (50 mL) and washed with water (50 mL) and brine (50 mL). The water layers were extracted with ether (50 mL) and the combined organics were dried with (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (0–2% EtOAc in pentane) gave the title compound (2.3 g, 8.1 mmol, 81%) as a clear oil. $R_f = 0.58$ (2% EtOAc in Pentane); ^1H NMR (400 MHz, CDCl_3) δ 6.96 (dm, 1 H, $J = 152.0$ Hz, H-3), 5.81 (dd, 1 H, $J = 15.6, 5.2$ Hz, H-2), 4.18 (q, 2 H, $J = 7.2$ Hz, $\text{CH}_2\text{-Ethyl}$), 2.19 (dm, 2 H, $J = 126.0$ Hz), 1.62–1.22 (m, 25 H, $\text{CH}_3\text{-Ethyl}$ and C-5 to C-15), 0.88 (t, 3 H, $J = 6.8$ Hz, H-16); ^{13}C NMR (101 MHz, CDCl_3) δ 166.9 (d, $J = 6.0$ Hz, C=O), 150.84 (dt, $J = 39.0, 17.0$ Hz, C-2), 149.65 (dd, $J = 41.0, 2.0$ Hz), 60.24 ($\text{CH}_2\text{-Ethyl}$), 32.33 (dd, $J = 41.0, 34.0$ Hz, C-4), 29.8–29.0 (m, $\text{CH}_2 \times 9$), 28.1 (dd, $J = 34.0, 2.0$ Hz, C-5), 22.8 (CH_2), 14.4 ($\text{CH}_3\text{-Ethyl}$), 14.3 (C-16); IR (neat): 2922, 2852, 1720, 1626, 1466, 1365, 1301, 1263, 1175, 1034, 977, 721 cm^{-1} ; HRMS calculated for $[\text{C}_{15}\text{-}^{13}\text{C}_3\text{H}_{34}\text{O}_2 + \text{H}]^+$: 286.2738, found 286.2733.

Ethyl-[3,4,5- $^{13}\text{C}_3$]-hexadecanoate (28). Ethyl (E)-[3,4,5- $^{13}\text{C}_3$]-hexadec-2-enoate **27** (2.20 g, 7.71 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, 0.41 g, 0.38 mmol, 0.05 eq). The reaction mixture was then stirred under a flow of hydrogen gas for 30 min 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 (50 mL) followed by removal of the solvents *in vacuo*. Purification by column chromatography (1% EtOAc in pentane) afforded the title compound as a clear oil (2.21 g, 7.32 mmol, 95%). $R_f = 0.58$ (2% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 4.12 (q, 2 H, $J = 7.2$ Hz, $\text{CH}_2\text{-Ethyl}$), 2.28 (m, 2 H, H-2), 1.61 (dm, 2 H, $J = 130.8$ Hz, H-3), 1.46–1.08 (m, 27 H, $\text{CH}_3\text{-Ethyl}$ and C-4 to C-15), 0.88 (t, 3 H, $J = 6.8$ Hz, H-16); ^{13}C NMR (101 MHz, CDCl_3) δ 174.0 (C=O), 62.0 ($\text{CH}_2\text{-Ethyl}$), 32.1 (C-2), 29.8–28.8 (m, C-4, C-5 and $\text{CH}_2 \times 10$), 25.4–24.7 (m, C-3), 22.8 (CH_2), 14.3 ($\text{CH}_3\text{-Ethyl}$), 14.2 (C-16); IR (neat): 2920, 2851, 1738, 1463, 1238, 1174, 1035, 733 cm^{-1} ; HRMS calculated for $[\text{C}_{15}\text{-}^{13}\text{C}_3\text{H}_{36}\text{O}_2 + \text{H}]^+$: 288.2894, found 288.2889.

[3,4,5- $^{13}\text{C}_3$]-palmitic acid (29). Ethyl-[3,4,5- $^{13}\text{C}_3$]-hexadecanoate **28** (2.10 g, 7.30 mmol, 1.0 eq) was dissolved in THF:EtOH:H₂O (1:1:1) (35 mL) and lithium hydroxide (0.52 g, 21.9 mmol, 3.0 eq) was added. The reaction was stirred at room temperature over night. The reaction mixture was then transferred to an extraction funnel with EtOAc (50 mL) and



washed with 1 M HCl (50 mL), water (50 mL) and brine (50 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics dried (Na₂SO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (5% EtOAc, 1% AcOH in pentane) gave **29** as a white solid (1.89 g, 6.94 mmol, 95%). *R*_f = 0.6 (10% EtOAc, 1% AcOH in pentane); ¹H NMR (400 MHz, CDCl₃) δ 11.40 (bs, 1 H, COOH), 2.35 (m, 2 H, H-2), 1.61 (dm, 2 H, *J* = 130.0 Hz, H-3), 1.52–1.06 (m, 24 H, C-4 to C-15), 0.88 (t, 3 H, *J* = 6.8 Hz, C-16); ¹³C NMR (100 MHz, CDCl₃) δ 180.1 (COOH), 32.1 (C-2), 29.9–28.7 (m, C-4, C-5 and CH₂ x10), 25.2–24.4 (m, C-3), 22.8 (CH₂), 14.3 (C-16); IR (neat); 2912, 2847, 1694, 1470, 1430, 1308, 1288, 941, 718, 679 cm⁻¹.

[3,4,5-¹³C₃]-palmitoyl chloride (30). [3,4,5-¹³C₃]-Palmitic acid (1.80 g, 6.93 mmol, 1.0 eq) was dissolved in dry DCM (72 mL), put under an atmosphere of argon, and cooled to 0 °C. Oxalyl chloride (1.2 mL, 14 mmol, 2 eq) was added followed by a drop of DMF. The reaction was then kept under a flow of argon and at room temperature. When gas evolution stopped (~ 2 h), the reaction was concentrated *in vacuo* giving the title product (1.90 g, 6.93 mmol, 100%). ¹H NMR (400 MHz, CDCl₃) δ 2.88 (m, 2 H, H-2), 1.70 (dm, 2 H, *J* = 130.5 Hz, H-3), 1.56–1.05 (m, 24 H, H-4 to H-15), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ¹³C NMR (100 MHz, CDCl₃) δ 166.4 (C=O), 32.0 (CH₂), 29.8–28.0 (m, C-4, C-5 and 8x CH₂), 25.15 (C-2), 24.14 (dd, *J* = 32.0, 1.5 Hz, C-3), 22.8 (CH₂), 14.2 (C-16); IR (neat); 2918, 2848, 2747, 1800, 1660, 1384, 1305, 1161, 1033, 908, 802 cm⁻¹.

D-erythro-sphingosine (31a). 3-*O*-Benzoyl-*N*-(*tert*-butoxycarbonyl)-D-erythro-sphingosine **23a** (90 mg, 0.18 mmol, 1.0 eq), was dissolved in 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 room temperature. The reaction was quenched with acetic acid (0.05 mL, 0.9 mmol), before concentration *in vacuo*. The residue was cooled to 0 °C before addition of water (0.66 mL) and 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 general procedure for HPLC-MS purifications) produced the title compound (41 mg, 0.1 mmol, 54%) as a TFA adduct. [α]_D²²: -2.0 (c = 0.5 MeOH); ¹H NMR (600 MHz, MeOD-*d*₄) δ 5.85 (m, 1 H, H-5), 5.47 (m, 1 H, H-4), 4.28 (m, 1 H, H-2), 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), 3.19 (dt, 1 H, *J* = 8.6, 4.4 Hz, H-2), 2.10 (q, 2 H, *J* = 7.1 Hz, H-6), 1.45–1.32 (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); ¹³C 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-4), 33.1, 30.81, 30.80 (2x CH₂), 30.77, 30.75, 30.65, 30.5, 30.4 (9x CH₂), 30.2 (C-7), 23.8 (CH₂), 14.4 (C-18); IR (neat): 3289, 2918, 2850, 1668, 1520, 1470, 1192, 1134, 986, 720 cm⁻¹; HRMS calculated for [C₁₈H₃₇NO₂ + H]⁺: 300.2897, found 300.2899.

[5,6,7,8,9-¹³C₅]-D-erythro-sphingosine (31b). [5,6,7,8,9-¹³C₅]-3-*O*-Benzoyl-*N*-(*tert*-butoxycarbonyl)-D-erythro-sphingosine **23b** (90 mg, 0.29 mmol, 1 eq.) was dissolved in methanol (10 mL) and sodium methoxide (30% in methanol) (19 µL, 0.14 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) (1.2 mL, 0.59 mmol, 2 eq) was added and the reaction was stirred over night at room temperature. The reaction was quenched with acetic acid (0.08 mL, 1.45 mmol, 5 eq), before concentration *in vacuo*. The residue was cooled to 0 °C before the addition of water (1 mL) and TFA (3 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 (52 mg, 0.17 mmol, 59%) as a TFA adduct. [α]_D²²: -2.0 (c = 0.5 MeOH); ¹H NMR (600 MHz, MeOD-*d*₄) δ 5.85 (dm, 1 H, *J* = 150 Hz, H-5), 5.47 (dt, 1 H, *J* = 15.6, 6.0 Hz, H-4), 4.28 (dd, 1 H, *J* = 11.4, 4.8 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, 8.3 Hz, H-1_b), 3.19 (dt, 1 H, *J* = 8.5, 4.3 Hz, H-2), 2.1 (dm, 2 H, *J* = 126.0 Hz, H-6), 1.56–1.20 (m, 22 H, H-7 to H-17), 0.90 (t, 3 H, *J* = 7.0 Hz, H-18); ¹³C NMR (151 MHz, MeOD-*d*₄) δ 135.6 (dd, *J* = 42.0, 3.0 Hz, C-5),

128.5 (dd, $J = 72.0, 3.0$ Hz), 71.0 (dd, $J = 5.5, 1.3$ Hz, C-3), 59.5 (C-1), 58.5 (d, $J = 3.0$ Hz, C-2), 33.8-32.9 (m, C-6 and CH₂), 30.9-29.8 (m, CH₂ x10), 23.7 (CH₂), 14.5 (C-18); IR (neat): 3287, 2914, 2847, 1661, 1526, 1470, 1198, 1136, 966, 721 cm⁻¹; HRMS calculated for [C₁₃¹³C₅H₃₇NO₂ + H]⁺: 305.2897, found 305.3065.

Ceramide (32a). See general procedure for the synthesis of the ceramides from sphingosine. Yield (20 mg, 37 μmol, 79%). $R_f = 0.48$ (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: -7.6 ($c = 1.0$ MeO:CHCl₃ 1:1); ¹H NMR (600 MHz, CDCl₃) δ 6.26 (d, 1 H, $J = 7.8$ Hz, NH), 5.78 (dt, 1 H, $J = 15.4, 7.0$ Hz, H-5), 5.53 (dd, 1 H, $J = 15.4, 6.5$ Hz, H-4), 4.31 (t, 1 H, $J = 4.7$ Hz, H-3), 3.95 (dd, 1 H, $J = 11.2, 3.8$ Hz, H-1a), 3.90 (m, 1 H, H-2), 3.70 (dd, 1 H, $J = 11.4, 3.6$ Hz, H-1b), 3.00-2.60 (bs, 2 H, 2x OH), 2.23 (t, 2 H, $J = 7.7$ Hz, H-2*), 2.05 (q, 2 H, $J = 7.2$ Hz, H-6), 1.63 (m, 2 H, H-3*), 140-1.21 (m, 46 H, H-7 to H-17 and H-4* to H-15*), 0.88 (t, 6 H, $J = 7.0$ Hz, H-18 and H-16*); ¹³C NMR (150 MHz, CDCl₃) δ 174.1 (C=O*), 134.4 (C-5), 128.9 (C-4), 74.5 (C-3), 62.4 (C-1), 54.7 (C-2), 37.0 (C-2*), 32.4 (C-6), 32.08, 29.86 x4, 29.85 x4, 29.82 x3, 29.80, 29.78, 29.67, 29.65, 29.53, 29.52 x2, 29.45, 29.39, 29.28, 25.92, 22.84 (CH₂ x24 C-7 to C-17 and C-3* to C-15*), 14.3 x2 (C-18 and C-16*); IR (neat): 3308, 2914, 2865, 1645, 1548, 1464, 1049, 959, 719 cm⁻¹; HRMS calculated for [C₃₄H₆₇NO₃ + H]⁺: 538.5121, found 538.5192.

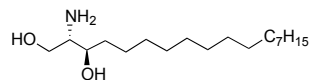
2-*N*-([3,4,5-¹³C]-hexadecanoyl)-sphingosine (32b). See general procedure for the synthesis of the ceramides from sphingosine. Yield (14 mg, 25 μmol, 71%). $R_f = 0.48$ (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: -8.0 ($c = 0.1$ MeOH:CHCl₃ 1:1); ¹H NMR (600 MHz, CDCl₃/MeOD-*d*₄) δ 6.26 (d, 1 H, $J = 7.8$ Hz, NH), 5.78 (dt, 1 H, $J = 15.4, 7.0$ Hz, H-5), 5.53 (dd, 1 H, $J = 15.4, 6.5$ Hz, H-4), 4.31 (t, 1 H, $J = 4.7$ Hz, H-3), 3.95 (dd, 1 H, $J = 11.2, 3.8$ Hz, H-1a), 3.90 (m, 1 H, H-2), 3.70 (dd, 1 H, $J = 11.4, 3.6$ Hz, H-1b), 3.00-2.60 (bs, 2 H, 2x OH), 2.23 (m, 2 H, H-2*), 2.05 (q, 2 H, $J = 7.2$ Hz, H-6), 1.63 (dm, 2 H, $J = 130$ Hz, H-3*), 1.45-1.15 (m, 46 H, H-7 to H-17 and H-4* to H-15*), 0.88 (t, 6 H, $J = 7.0$ Hz, H-18 and H-16*); ¹³C NMR (151 MHz, CDCl₃/MeOD-*d*₄) δ 174.1 (C=O*), 134.4 (C-5), 128.9 (C-4), 74.8 (C-3), 62.6 (C-1), 54.7 (C-2), 37.0 (d, $J = 35.0$ Hz, C-2*), 32.4 (C-6), 32.0, 29.91, 29.85-29.38 (m), 26.15-25.79 (m), 22.84 (CH₂ x24 C-7 to C-17 and C-3* to C-15*), 14.3x2 (C-18 and C-16*); IR (neat): 3293, 2914, 2847, 1636, 1547, 1465, 1038, 972, 721 cm⁻¹; HRMS calculated for [C₃₁¹³C₅H₆₇NO₃ + H]⁺: 541.5121, found 541.5293.

2-*N*-(hexadecanoyl)-[5,6,7,8,9-¹³C]-sphingosine (32c). See general procedure for the synthesis of the ceramides from sphingosine. Yield (12 mg, 22 μmol, 73%). $R_f = 0.48$ (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: -7.2 ($c = 0.25$ MeOH:CHCl₃ 1:1); ¹H NMR (600 MHz, CDCl₃) δ 6.31 (d, 1 H, $J = 7.8$ Hz, NH), 5.76 (dm, 1 H, $J = 150.0$ Hz, H-5), 5.53 (m, 1 H, H-4), 4.30 (m, 1H, H-3), 3.95 (m, 1 H, H-1a), 3.90 (m, 1 H, H-2), 3.70 (m, 1 H, H-1a), 3.00-2.60 (bs, 2 H, 2x OH), 2.22 (t, 2 H, $J = 7.9$ Hz, H-2*), 2.05 (dm, 2 H, $J = 124.0$ Hz, H-6), 1.63 (m, 2 H, H-3*), 1.52-1.13 (m, 46 H, H-7 to H-17 and H-4* to H-15*), 0.88 (t, 6 H, $J = 7.0$ Hz, H-18 and H-16*); ¹³C NMR (151 Hz, CDCl₃) δ 174.2 (C=O*), 133.0 (d, $J = 43.0$ Hz, C-5), 129.1 (d, $J = 72.0$ Hz, C-4), 74.7 (d, $J = 20.8$ Hz, C-3), 62.6 (C-1), 54.6 (d, $J = 2.6$ Hz, C-2), 37.0 (C-2*), 32.8-32.0 (m, C-6 and CH₂), 29.9-29.0 (m), 28.25-27.74 (m), 25.92 (m), 22.84 (CH₂ x24 C-7 to C-17 and C-3* to C-15*), 14.3 x2 (C-18 and C-16*); IR (neat): 3300, 2914, 2847, 1701, 1635, 1547, 1464, 1124, 970, 719 cm⁻¹; HRMS calculated for [C₂₉¹³C₅H₆₇NO₃ + H]⁺: 543.5121, found 543.5358.

2-*N*-([3,4,5-¹³C]-hexadecanoyl)-[5,6,7,8,9-¹³C]-sphingosine (32d). See general procedure for the synthesis of the ceramides from sphingosine. Yield (18 mg, 33 μmol, 81%). $R_f = 0.48$ (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: -7.0 ($c = 0.33$ MeOH:CHCl₃ 1:1); ¹H NMR (600 MHz, CDCl₃) δ 6.26 (d, 1 H, $J = 7.8$ Hz, NH), 5.76 (dm, 1 H, $J = 150.0$ Hz, H-5), 5.53 (m, 1 H, H-4), 4.31 (m, 1H, H-3), 3.95 (dd, 1 H, $J = 11.2, 4.0$ Hz, H-1a), 3.90 (m, 2 H, H-2), 3.70 (dd, 1 H, $J = 11.4, 3.2$ Hz, H-1b), 3.00-2.60 (bs, 2 H, 2x OH), 2.23 (m, 2 H, H-2*), 2.05 (dm, 2 H, $J = 124.0$ Hz, H-6), 1.63 (dm, 2 H, $J = 130$ Hz, H-3*), 1.50-1.15 (m, 46 H, H-7 to H-17 and H-4* to H-15*), 0.88 (t, 6 H, $J = 7.0$ Hz, H-18 and H-16*); ¹³C NMR(151 MHz, CDCl₃) δ 174.1

(C=O*), 134.4 (d, $J = 43.0$ Hz, C-5), 128.8 (d, $J = 72.0$ Hz, C-4), 74.5 (d, $J = 20.8$ Hz, C-3), 62.6 (C-1), 54.6 (d, $J = 2.6$ Hz C-2), 37.0 (d, $J = 35.0$ Hz, C-2*), 32.8-32.0 (m, C-6 and CH₂), 29.9-29.0 (m), 28.2-27.7 (m), 26.2-25.6 (m), 21.7 (CH₂ x24 C-7 to C-17 and C-3* to C-15*), 14.3 x2 (C-18 and C-16*); IR (neat): 3294, 2914, 2847, 1699, 1636, 1547, 1464, 1040, 970, 719 cm⁻¹; HRMS calculated for [C₂₆¹³C₈H₆₇NO₃ + H]⁺: 546.5121, found 546.5461.

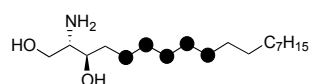
Sphinganine (33a). 3-*O*-Benzoyl-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine **23a** (36.8 mg, 0.07 mmol, 1.0



eq), was dissolved in methanol (2.4 mL), and sodium methoxide (30% in methanol) (4.6 μL, 0.035 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) (0.28 mL, 0.14 mmol, 2.0 eq), was added and the reaction was stirred over night at room temperature. The reaction was quenched with acetic acid (0.019 mL, 0.35 mmol, 5.0 eq), before concentration *in vacuo*. The residue was co-evaporated once with toluene (4.0 mL) and then dissolved in EtOAc (1 mL). The solution was purged with argon, before addition of platinum dioxide (1.5 mg, 0.007 mmol, 0.1 eq). The reaction mixture was then stirred under a flow of hydrogen gas for 30 min and then left under a hydrogen atmosphere over night. The platinum dioxide residue was removed by filtration over a plug of Celite and then rinsed with EtOAc followed by concentration *in vacuo*. The residue was cooled to 0 °C before the addition of water (1 mL) and TFA (2 mL). The reaction was stirred for 2 minutes at 0 °C and then diluted with toluene (4 mL) and concentrated *in vacuo*. Purification by HPLC-MS (52-62% B, following the general procedure for HPLC-MS purifications) produced the title compound (10 mg, 33 μmol, 47%) as a TFA adduct. $[\alpha]_D^{22}$: -7.0 (c = 0.1 MeOH); ¹H NMR (600 MHz, MeOD-*d*₄) δ 3.83 (dd, 1 H, $J = 11.6, 4.0$ Hz, H-1_a), 3.77 (dt, 1 H, $J = 8.4, 4.2$ Hz, H-3), 3.70 (dd, 1 H, $J = 11.5, 8.7$ Hz, H-1_b), 3.19 (dt, 1 H, $J = 8.3, 3.9$ Hz, H-2), 1.55-1.22 (m, H 28, H-4 to H-17), 0.90 (t, 3 H, $J = 7.0$ Hz, H-18); ¹³C NMR (151 MHz, MeOD-*d*₄) δ 70.3 (C-3), 58.8 (C-1), 58.4 (C-2), 34.2 (C-4), 33.1, 30.84 (x4), 30.78, 30.77, 30.74, 30.70, 30.57, 30.49, 27.0, 23.8 (CH₂ x13, C-5 to C-17), 14.5 (C-18); IR (neat): 3150, 2914, 2849, 1676, 1207, 1186, 1153, 1126, 1053, 840, 800, 721 cm⁻¹; HRMS calculated for [C₁₈H₃₉NO₂ + H]⁺: 302.2981, found 302.3054.

[5,6,7,8,9-¹³C₅]-Sphinganine (33b). [5,6,7,8,9-¹³C₅]-3-*O*-Benzoyl-*N*-(*tert*-butoxycarbonyl)-*D*-erythro-sphingosine



23b (81.4 mg, 0.16 mmol, 1.0 eq), was dissolved in methanol (5.5 mL), and sodium methoxide (30% in methanol) (10 μL, 0.08 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)

(0.64 mL, 0.32 mmol, 2.0 eq), was added and the reaction was stirred over night at room temperature. The reaction was quenched with acetic acid (4.4 μL, 0.8 mmol, 5.0 eq), before concentration *in vacuo*. The residue was co-evaporated once with toluene (10 mL) and then dissolved in EtOAc (2 mL). The solution was purged with argon, before addition of platinum dioxide (3.6 mg, 0.016 mmol, 0.1 eq). The reaction mixture was then stirred under a flow of hydrogen gas for 30 min and then left under a hydrogen atmosphere over night. The platinum dioxide residue was removed by filtration over a plug of Celite and then rinsed with EtOAc followed by concentration *in vacuo*. The residue was cooled to 0 °C before the addition of water (0.5 mL) and TFA (1.5 mL). The reaction was stirred for 2 minutes at 0 °C and was then diluted with toluene (10 mL) and concentrated *in vacuo*. Purification by HPLC-MS (52-62% B, following the general procedure for HPLC-MS purifications) produced the title compound (25 mg, 83 μmol, 52%) as a TFA adduct. $[\alpha]_D^{22}$: -7.5 (c = 0.1 MeOH); ¹H NMR (600 MHz, MeOD-*d*₄) δ 3.83 (dd, 1 H, $J = 11.5, 4.0$ Hz, H-1_a), 3.76 (dt, 1 H, $J = 8.3, 4.3$ Hz, H-3), 3.69 (dd, 1 H, $J = 11.5, 8.8$ Hz, H-1_b), 3.18 (dt, 1 H, $J = 8.9, 4.0$ Hz, H-2), 1.65-1.15 (m, 28 H, H-4 to H-17), 0.90 (t, 3 H, $J = 7.0$ Hz, H-18); ¹³C NMR (151 MHz, MeOD-*d*₄) δ 70.3 (C-3), 58.6 (C-1), 58.4 (C-2), 34.1 (d, $J = 34.7$ Hz, C-4), 33.1 (CH₂), 31.1-30.2 (m, CH₂ x10), 27.3-26.7 (m, CH₂), 23.8 (CH₂), 14.5 (C-18); IR (neat): 3120, 2914, 2847, 1676, 1206, 1186, 1153, 1130, 840, 800, 723 cm⁻¹; HRMS calculated for [C₁₈H₃₉NO₂ + H]⁺: 307.2981, found 307.3222.

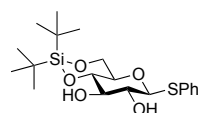
Dihydroceramide (34a). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (12 mg, 22 μ mol, 68%); R_f = 0.50 (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: +4.5 (c = 0.15 MeOH:CHCl₃ 1:1); ^1H NMR (600 MHz, CDCl₃, 318 $^\circ\text{K}$) δ 6.36 (d, 1 H, J = 7.6 Hz, NH), 4.01 (d, 1 H, J = 11.3 Hz, H-1_a), 3.83 (m, 1 H, H-2), 3.80-3.72 (m, 2 H, H-1_b and H-3), 2.90-2.50 (bs, 2 H, 2x OH), 2.23 (t, 2 H, J = 7.4 Hz, H-2*), 1.68-1.59 (m, 4 H, H-4 and H-3*), 1.59-1.45 (m, 2 H, H-5 and H-4*), 1.38-1.19 (m, 46 H, H-6 to H-17 and H-5* to H-15*), 0.88 (t, 6 H, J = 7.2 Hz, H-18 and H-16*); ^{13}C NMR (151 MHz, CDCl₃, 318 $^\circ\text{K}$) δ 173.7 (C=O*), 74.4 (C-3), 62.7 (C-1), 54.2 (C-2), 37.1 (C-2*), 34.7 (C-4), 32.10, 29.86 x4, 29.84 x3, 29.82 x3, 29.79 x2, 29.75 x3, 29.72, 29.71, 29.67, 29.66, 29.52, 29.51 x2, 29.48, 22.84 (CH₂ x26 C-4 to C-17 and C-4* to C-15*), 14.2 x2 (C-18 and C-16*); IR (neat): 3395, 2914, 2849, 1738, 1630, 1570, 1470, 1047, 719 cm⁻¹; HRMS calculated for [C₃₄H₆₇NO₃ + H]⁺: 540.5121, found 540.5347.

2-*N*-([3,4,5-¹³C₃]-hexadecanoyl)-sphinganine (34b). See general procedure for the synthesis of the ceramides from the sphingosine (15 mg, 27 μ mol, 74%); R_f = 0.50 (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: +4.8 (c = 0.1 MeOH:CHCl₃ 1:1); ^1H NMR (600 MHz, CDCl₃, 318 $^\circ\text{K}$) δ 6.26 (d, 1 H, J = 7.6 Hz, NH), 4.01 (d, 1 H, J = 11.3 Hz, H-1_a), 3.83 (m, 1 H, H-2), 3.80-3.72 (m, 2 H, H-1_b and H-3), 2.80-2.40 (bs, 2 H, 2x OH), 2.23 (m, 2 H, H-2*), 1.80-1.10 (m, 54 H, H-4 to H-17 and H-3* to H-15*), 0.88 (t, 6 H, J = 7.2 Hz, H-18 and H-16*); ^{13}C NMR (151 MHz, CDCl₃, 318 $^\circ\text{K}$) δ 173.7 (C=O*), 74.5 (C-3), 62.7 (C-1), 54.1 (C-2), 37.1 (d, J = 34.0 Hz, C-2*), 34.8 (C-4), 32.09, 29.9-29.3 (m), 29.18, 26.2-25.8 (m), 25.67, 22.83 (CH₂ x26 C-4 to C-17 and C-4* to C-15*), 14.2 x2 (C-18 and C-16*); IR (neat): 3394, 2914, 2849, 1738, 1630, 1570, 1470, 1049, 719 cm⁻¹; HRMS calculated for [C₃₁¹³C₃H₆₇NO₃ + H]⁺: 543.5121, found 543.5442.

2-*N*-(hexadecanoyl)-[5,6,7,8,9-¹³C₅]-sphinganine (34c). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (14 mg, 25 μ mol, 65%); R_f = 0.50 (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: +5.2 (c = 0.25 MeOH:CHCl₃ 1:1); ^1H NMR (600 MHz, CDCl₃, 318 $^\circ\text{K}$) δ 6.26 (d, 1 H, J = 7.6 Hz, NH), 4.01 (d, 1 H, J = 11.3 Hz, H-1_a), 3.83 (m, 1 H, H-2), 3.80-3.72 (m, 2 H, H-1_b and H-3), 2.70-2.40 (bs, 2 H, 2x OH) 2.22 (t, 2 H, J = 7.9 Hz, H-2*), 1.65-1.15 (m, 54 H, H-4 to H-17 and H-3* to H-15*), 0.89 (t, 6 H, J = 7.2 Hz, H-18 and H-16*); ^{13}C NMR (151 MHz, CDCl₃, 318 $^\circ\text{K}$) δ 173.7 (C=O*), 74.4 (C-3), 62.7 (C-1), 54.1 (d, J = 2.6 Hz, C-2), 37.1 (C-2*), 34.8 (d, J = 35.0 Hz, C-4), 34.0, 32.10, 29.9-29.5 (m), 26.4-25.8 (m), 22.8 (CH₂ x26 C-4 to C-17 and C-4* to C-15*), 14.2 x2 (C-18 and C-16*); IR (neat): 3399, 2914, 2849, 1630, 1568, 1470, 1047, 717 cm⁻¹; HRMS calculated for [C₂₉¹³C₅H₆₇NO₃ + H]⁺: 545.5121, found 545.5515.

2-*N*-([3,4,5-¹³C₃]-hexadecanoyl)-[5,6,7,8,9-¹³C₅]-sphinganine (34d). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (18 mg, 33 μ mol, 66%); R_f = 0.50 (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: +5.0 (c = 0.25 MeOH:CHCl₃ 1:1); ^1H NMR (600 MHz, CDCl₃, 318 $^\circ\text{K}$) δ 6.26 (d, 1 H, J = 7.6 Hz, NH), 4.01 (d, 1 H, J = 11.3 Hz, H-1_a), 3.83 (m, 1 H, H-2), 3.80-3.72 (m, 2 H, H-1_b and H-3), 2.55 (bs, 1 H, OH), 2.45 (bs, 1 H, OH), 2.23 (m, 2 H, H-2*), 1.72-1.12 (m, 54 H, H-4 to H-17 and H-3* to H-15*), 0.88 (t, 6 H, J = 7.2 Hz, H-18 and H-16*); ^{13}C NMR (151 MHz, CDCl₃, 318 $^\circ\text{K}$) δ 173.7 (C=O*), 74.4 (C-3), 62.7 (C-1), 54.1 (d, J = 2.6 Hz, C-2), 37.1 (d, J = 35.0 Hz, C-2*), 34.8 (d, J = 35.0 Hz, C-4), 34.0 (m), 32.1, 30.0-29.3 (m), 29.18, 26.4-25.7 (m), 25.67, 22.8 (CH₂ x26 C-4 to C-17 and C-4* to C-15*), 14.2 (C-18 and C-16*); IR (neat): 3390, 2914, 2849, 1726, 1630, 1572, 1470, 1047, 716 cm⁻¹; HRMS calculated for [C₂₆¹³C₈H₆₇NO₃ + H]⁺: 548.5121, found 548.5611.

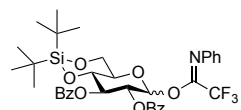
Phenyl-4,6-*O*-(di-*tert*-butylsilanediyl)-1-thio- β -D-glucosylpyranoside (35b). Phenyl-1-thio- β -D-glucoside (35a) (1.85 g, 6.8 mmol, 1.05 eq) was dissolved in dry DMF (27 mmol) under an Argon atmosphere. This solution was cooled down to -40 $^\circ\text{C}$ before drop wise addition of di-*tert*-butylsilylbis(trifluoromethanesulfonate) (2.1 mL, 6.5 mmol, 1 eq). The resulting reaction was stirred at -40 $^\circ\text{C}$ for 30 minutes followed by addition of pyridine (1.58 mL,



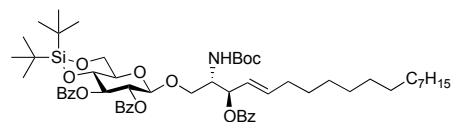
19.5 mmol, 3 eq). The reaction was stirred for an additional 15 minutes and then transferred to an extraction funnel with diethyl ether (50 mL). The organics were washed with water (2x 100 mL) and brine (100 mL). The aqueous layers were extracted with diethyl ether (50 mL) and the combined organics were dried (Na_2SO_4) filtered and concentrated *in vacuo*. Purification by silica column chromatography (10% Et₂O in petroleum ether) yielded the title compound (2.16 g, 5.2 mmol, 77%). R_f = 0.8 (50% EtOAc in petroleum ether); $[\alpha]_D^{22}$: -37 (C = 1.0 CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.53-7.50 (m, 2 H, H_{arom}), 7.33-7.28 (m, 3 H, H_{arom}), 4.61 (d, 1 H, J = 10.0 Hz, H-1), 4.21 (dd, 1 H, J = 10.3, 5.2 Hz, H-6_a), 3.90 (t, 1 H, J = 10.2 Hz, H-6_b), 3.69 (t, 1 H, J = 9.2 Hz, H-3), 3.61 (t, 1 H, J = 8.7 Hz, H-4), 3.49 (m, 2 H, H-2 and H-5), 3.25 (bs, 2 H, OH), 1.05 (s, 3 H, H-*t*Bu-Si), 0.98 (s, 3 H, H-*t*Bu-Si); ^{13}C NMR (101 MHz, CDCl_3) δ 132.9 (C_{q-arom}), 131.9 (C_{q-arom}), 129.1 (CH_{arom} x2), 128.3 (CH_{arom} x2), 88.6 (C-1), 77.9 (C-3), 76.5 (C-4), 74.6 (C-5), 71.9 (C-2), 66.2 (C-6), 27.5 (CH_{3-t}Bu-Si), 27.1 (CH_{3-t}Bu-Si), 22.8 (C_{q-t}Bu-Si), 20.0 (C_{q-t}Bu-Si); IR (neat): 3380, 2931, 2858, 1472, 1055, 823, 731, 651 cm^{-1} ; HRMS: calculated. For $[\text{C}_{20}\text{H}_{32}\text{O}_5\text{Si} + \text{H}]^+$ 413.1740, found 413.1801.

Phenyl 2,3-di-O-benzoyl-4,6-O-(di-*tert*-butylsilanediyl)-1-thio- β -D-glucopyranoside (35c). Phenyl- 4,6-O-(di-*tert*-butylsilanediyl)-1-thio- β -D-glucosylpyranoside (**35b**) (2.16 g, 5.2 mmol, 1.0 eq) was dissolved in dry pyridine (13 mL) and benzoyl chloride (3.25 mL, 28.0 mmol, 2.4 eq) was added. The reaction was stirred until TLC showed full conversion to a higher running product and was then quenched with methanol (1 mL) and concentrated *in vacuo*. The residue was dissolved in EtOAc (50 mL) and washed with 1 N HCl (50 mL), 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 (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (10% Et₂O in petroleum ether) afforded the title compound (3.18 g, 5.12 mmol, 98%). $[\alpha]_D^{22}$: +47.2 (C = 1.0 CHCl_3); R_f = 0.7 (15% EtOAc in petroleum ether); ^1H NMR (400 MHz, CDCl_3) δ 8.00-7.92 (m, 4 H, H_{arom}), 7.55-7.26 (m, 11 H, H_{arom}), 5.59 (t, 1 H, J = 9.5 Hz H-3), 5.39 (t, 1 H, J = 9.8 Hz, H-2), 4.99 (d, 1 H, J = 10.0 Hz, H-1), 4.31 (dd, 1 H, J = 10.0, 5.1 Hz, H-6_a), 4.10 (t, 1 H, J = 9.2 Hz, H-4), 4.01 (t, 1 H, J = 10.0 Hz, H-6_b), 3.69 (td, 1 H, J = 10.0, 5.2 Hz, H-5), 0.973 (s, 9 H, CH_{3-t}Bu-Si), 0.967 (s, 9H, CH_{3-t}Bu-Si); ^{13}C NMR (101 MHz, CDCl_3) δ 165.8 (C=O_{Bz}), 165.1 (C=O_{Bz}), 134.5, 133.2, 132.9, 132.1, 130.5, 129.8, 129.7, 129.6, 128.3, 129.0, 128.8, 128.3, 128.2 (CH_{arom}), 87.0 (C-1), 76.2 (C-3), 75.1 (C-5), 74.9 (C-4), 70.6 (C-2), 66.1 (C-6), 27.3 (CH_{3-t}Bu-Si), 26.9 (CH_{3-t}Bu-Si), 22.5 (C_{q-t}Bu-Si), 19.9 (C_{q-t}Bu-Si); IR (neat): 2959, 2932, 2883, 2858, 1732, 1271, 1177, 1126, 827, 708 cm^{-1} ; HRMS: calculated for $[\text{C}_{34}\text{H}_{40}\text{O}_7\text{Si} + \text{H}]^+$ 621.2344; found 621.2337.

2,3-di-O-benzoyl-4,6-O-(di-*tert*-butylsilanediyl)- α - β -D-glucopyranose (35d). Phenyl 2,3-di-O-benzoyl-4,6-O-(di-*tert*-butylsilanediyl)-1-thio- β -D-glucopyranoside (**35c**) (6.27 g, 10.7 mmol, 1.0 eq) was dissolved in DCM (100 mL). *N*-iodosuccinimide (4.81 g, 21.4 mmol, 2.0 eq) was added at 0 °C, before addition of trifluoroacetic acid (0.82 mL, 10.7 mmol, 1.0 eq). The reaction was left stirring under exposure to the atmosphere until TLC showed full conversion. The mixture was transferred to an extraction funnel with EtOAc (200 mL) and washed with sodium thiosulfate (20% aq., 200 mL), sat. aq. NaHCO_3 (200 mL), and brine (200 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 silica gel column chromatography (5% EtOAc in petroleum ether) afford the title compound (6.63 g, 10.5 mmol, 98%, 5:3 α : β). R_f = 0.1 (10% EtOAc in petroleum ether); ^1H NMR (400 MHz, CDCl_3) δ 8.10 (m, 1 H, H_{arom}), 8.03-7.94 (m, 6 H, H_{arom}), 7.60 (m, 0.6 H, H_{arom}), 7.55-7.43 (m, 4.5 H, H_{arom}), 7.41-7.32 (m, 6.3 H, H_{arom}), 5.92 (t, 1 H, J = 9.1 Hz, H-3), 5.66-5.61 (m, 1.6 H, H-2), 5.22 (dd, 0.6 H, J = 9.5, 7.8 Hz), 5.18 (dd, 1 H, J = 10.3, 3.9 Hz), 4.97 (d, 0.6 H, J = 8.1 Hz), 4.31-4.13 (m, 3.2 H), 4.12-4.06 (m, 2 H), 4.03-3.91 (m, 2 H), 3.67 (td, 0.6 H, J = 10.4, 5.2 Hz), 1.00-0.97 (m, 25.2 H); ^{13}C NMR (101 MHz, CDCl_3) δ 167.1, 166.2, 166.15, 166.0 (C=O_{Bz}), 133.73, 133.69, 133.50, 133.20, 133.00, 130.27, 130.11, 130.09, 130.02, 129.72, 129.69, 129.16, 128.95, 128.54, 128.46, 128.40 (CH_{arom}), 96.4, 90.9, 75.6, 75.3, 74.51, 74.47, 72.4, 74.3, 71.2, 66.73, 66.52, 66.32, 27.44 (CH_{3-t}Bu-Si), 27.41 (CH_{3-t}Bu-Si), 27.00 (CH_{3-t}Bu-Si), 26.96 (CH_{3-t}Bu-Si), 22.75 (C_{q-t}Bu-Si), 22.72 (C_{q-t}Bu-Si), 20.08 (C_{q-t}Bu-Si), 20.05 (C_{q-t}Bu-Si); IR (neat): 3431, 2934, 2859, 1728, 1277, 1177, 1070, 827, 708 cm^{-1} ; HRMS: calculated for $[\text{C}_{26}\text{H}_{38}\text{O}_8\text{Si} + \text{H}]^+$ 529.2259, found 529.2256.

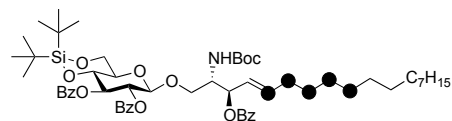
2,3-di-O-benzoyl-4,6-O-(di-*tert*-butylsilanediyl)-1-O-(*N*-[phenyl]-trifluoroacetimidoyl)- α/β -D-glucopyranose**(35d).** 2,3-di-O-benzoyl-4,6-O-(di-*tert*-butylsilanediyl)- α/β -D-glucopyranose (**35d**)

(1.71 g, 3.45 mmol, 1.0 eq) was dissolved in acetone (20 mL) and cooled down to 0 °C. Cesium carbonate (1.69 g, 5.18 mmol, 1.5 eq) was added followed by chloro *N*-phenyl-trifluoroimide (0.78 mL, 5.18 mmol, 1.5 eq), and the reaction was stirred at 0 °C for 2 hours. The reaction mixture was filtered and concentrated *in vacuo*. Purification by silica gel column chromatography, using silica gel that was neutralized by running an eluent of 3% Et₃N in petroleum ether (100 mL) through the column (0-5% EtOAc, 20% DCM in petroleum ether) produced the title compound (1.93 g, 2.76 mmol, 80%,). *R*_f = 0.1 (10% EtOAc in petroleum ether); ¹H NMR (400 MHz, CDCl₃) δ 8.05-8.00 (m, 4 H, H_{arom}), 7.54-7.44 (m, 2 H, H_{arom}), 7.41-7.31 (m, 4 H, H_{arom}), 7.28 (m, 1 H, H_{arom}), 7.11 (t, 2 H, *J* = 8.0 Hz, H_{arom}), 7.00 (t, 1 H, *J* = 8.0 Hz, H_{arom}), 6.74 (m, 1 H, H-1), 6.45 (m, 1 H, H-3), 5.98 (m, 1 H, H-4), 5.50 (m, 1 H, H-2), 4.32-4.20 (m, 2 H, H-5 and H-6_a), 4.00 (m, 1 H, H-6_b), 1.05-0.97 (m, 18 H, H_{tBu-Si}); ¹³C NMR (101 MHz, CDCl₃) δ 165.55, 165.49 C=O_{Bz}, 142.96 (C=N), 133.68, 133.10, 130.00, 129.86, 129.70, 129.65, 128.82, 128.67, 128.63, 128.49, 128.40 (C_{arom}), 119.21 (C-1), 75.00 (C-2), 72.08 (C-4), 70.53 (C-3), 69.27 (C-5), 66.29 (C-6), 27.31 (C_{q-tBu-Si}), 26.83 (C_{q-tBu-Si}), 22.65 (C_{q-tBu-Si}), 20.00 (C_{q-tBu-Si}); IR (neat): 2959, 2936, 2860, 1728, 1273, 1211, 995, 766, 710 cm⁻¹; HRMS: calculated for [C₃₆H₄₀F₃NO₆Si + H]⁺ 700.2555, found 700.2549.

Glucosyl sphingosine (36a). 2,3-di-O-Benzoyl-4,6-O-(di-*tert*-butylsilanediyl)-1-O-(*N*-[phenyl]-trifluoroacetimidoyl) -

α/β -D-glucopyranose **35** (0.325 g, 0.465 mmol, 1.3 eq) and sphingosine acceptor **23a** (180 mg, 0.357 mmol, 1.0 eq) were co-evaporated twice with toluene (10 mL) and then dissolved in anhydrous DCM (4 mL). Activated molsieves (3 Å) were added and the mixture was stirred for 1 h at room

temperature, cooled to 0 °C before the addition of BF₃OEt₂ (44 μ L, 0.36 mmol, 1.0 eq). The reaction was stirred until TLC showed a lower running spot (debocylation of the sphingosine acceptor) (~ 1 h). The reaction mixture was transferred to an extraction funnel with EtOAc (50 mL), and washed with sat. aq. NaHCO₃ (50 mL) and brine (50 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were dried (Na₂SO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (2-5% Et₂O, 20% DCM in petroleum ether) produced the title compound as an amorphous solid (177 mg, 0.17 mmol, 49%); *R*_f = 0.45 (10% Et₂O, 20% DMC in petroleum ether); [α]_D²²: +6.8 (c = 0.1 CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.03-7.95 (m, 6H, H_{arom}), 7.56-7.48 (m, 3 H, H_{arom}), 7.45-7.35 (m, 6 H, H_{arom}), 5.79 (m, 1 H, H-5_{sp}), 5.56 (t, 1 H, *J* = 9.4 Hz, H-3), 5.50-5.41 (m, 2 H, H-3_{sp} and H-4_{sp}), 5.35 (dd, 1 H, *J* = 10.4, 7.8 Hz, H-2), 4.80 (d, 1 H, *J* = 9.5 Hz, NH), 4.68 (d, 1 H, *J* = 7.8 Hz, H-1), 4.11-4.02 (m, 3 H, H-4, H-1_{a-sp} and H-2_{sp}), 3.97 (dd, 1 H, *J* = 10.2, 4.5 Hz, H-6_a), 3.74 (t, 1 H, *J* = 10.2 Hz, H-6_b), 3.62 (m, 1 H, H-1_{b-sp}), 3.55 (m, 1 H, H-5), 1.96 (q, 2 H, *J* = 6.8 Hz, H-6_{sp}), 1.34 (s, 9 H, CH_{3-tBu-Boc}), 1.32-1.18 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.95 (s, 18 H, CH_{3-tBu-Si}), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18_{sp}); ¹³C NMR (101 MHz, CDCl₃) δ 165.9, 165.4, 165.1 (C=O_{Bz} x3), 155.4 (C=O_{Boc}), 137.6 (C-5_{sp}), 133.3, 133.1, 133.0 (CH_{arom} x3), 130.6 (C_{q-arom}), 129.9 (CH_{arom} x2), 129.8 (C_{q-arom}), 129.7 (CH_{arom} x4), 129.5 (C_{q-arom}), 128.5 (CH_{arom} x2), 128.4 (CH_{arom} x4), 124.7 (C-4_{sp}), 101.4 (C-1), 79.5 (C_{q-Boc}), 75.1 (C-3), 74.8 (C-4), 74.4 (C-3_{sp}), 72.1 (C-2), 70.8 (C-5), 67.9 (C-6), 66.0 (C-1_{sp}), 52.4 (C-2_{sp}), 32.4 (C-6_{sp}), 32.0, 29.80 (x4), 29.71, 29.59, 29.48, 29.35, 28.92 (CH_{2-sp} x10), 28.4 (CH_{3-tBu-Boc}), 27.4, 26.9 (CH_{3-tBu-Si} x2), 22.8 (C_{q-tBu-Si}), 22.6 (CH_{2-sp}), 20.0 (C_{q-tBu-Si}), 14.3 (C-18_{sp}); IR (neat): 3070, 2958, 2924, 2854, 1728, 1271, 1174, 1103, 1070, 709 cm⁻¹; HRMS calculated for [C₅₈H₈₃NO₁₂Si + Na]⁺: 1036.5685, found 1036.5584.

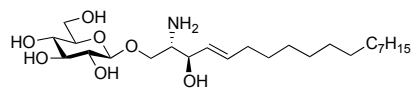
[5,6,7,8,9-¹³C₅]-glucosyl sphingosine (36b). 2,3-di-O-Benzoyl-4,6-O-(di-*tert*-butylsilanediyl)-1-O-(*N*-[phenyl]-

trifluoroacetimidoyl)- α/β -D-glucopyranose **35** (0.27 g, 0.4 mmol, 1.5 eq) and sphingosine acceptor **23b** (137 mg, 0.27 mmol, 1.0 eq) were co-evaporated twice with toluene (10 mL) and then dissolved in anhydrous DCM (3 mL). Activated molsieves (3 Å) were added and the mixture

was stirred for 1 h at room temperature and then cooled to 0 °C, before addition of BF₃OEt₂ (35 μ L, 0.27 mmol,

1.0 eq). The reaction was stirred until TLC showed a lower running spot (debenzylation of sphingosine acceptor) (~ 1 h). The reaction mixture was transferred to an extraction funnel with EtOAc (40 mL), and washed with sat. aq. NaHCO₃ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na₂SO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (2-5% Et₂O, 20% DCM in petroleum ether) produced the title compound as an amorphous solid (147 mg, 0.145 mmol, 54%). *R*_f = 0.45 (10% Et₂O, 20% DMC in petroleum ether); [α]_D²²: +6.0 (c = 0.1 CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.03-7.95 (m, 6H, H_{arom}), 7.57-7.48 (m, 3 H, H_{arom}), 7.45-7.34 (m, 6 H, H_{arom}), 5.79 (dm, 1 H, *J* = 151.2 Hz, H-5_{sp}), 5.55 (t, 1 H, *J* = 9.4 Hz, H-3), 5.50-5.41 (m, 2 H, H-3_{sp} and H-4_{sp}), 5.35 (dd, 1 H, *J* = 10.4, 7.8 Hz, H-2), 4.79 (d, 1 H, *J* = 8.9 Hz, NH), 4.67 (d, 1 H, *J* = 7.8 Hz, H-1), 4.11-4.02 (m, 3 H, H-4, H-1_{a-sp} and H-2_{sp}), 3.98 (dd, 1 H, *J* = 10.2, 4.5 Hz, H-6_a), 3.74 (t, 1 H, *J* = 10.2 Hz, H-6_b), 3.62 (m, 1 H, H-1_{b-sp}), 3.55 (m, 1 H, H-5), 1.96 (dm, 2 H, *J* = 126.2, H-6_{sp}), 1.34-1.10 (m, 31 H, CH₃-tBu-Boc and H-7_{sp} to H-17_{sp}), 0.95 (s, 18 H, CH₃-tBu-Si), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18_{sp}); ¹³C NMR (101 MHz, CDCl₃) δ 166.0, 165.4, 165.1 (C=O_{Bz} x3), 155.4 (C=O_{Boc}), 137.6 (d, *J* = 42.6 Hz, C-5_{sp}), 133.3, 133.1, 133.0 (CH_{arom} x3), 130.6 (C_{q-arom}), 129.9 (CH_{arom} x2), 129.8 (C_{q-arom}), 129.7 (CH_{arom} x4), 129.5 (C_{q-arom}), 128.5 (CH_{arom} x2), 128.4 (CH_{arom} x4), 124.7 (d, *J* = 71.2 Hz, C-4_{sp}), 101.4 (C-1), 79.5 (C_{q-Boc}), 75.1 (C-3), 74.8 (C-4), 74.4 (d, *J* = 5.2 Hz, C-3_{sp}), 72.1 (C-2), 70.8 (C-5), 67.9 (C-6), 66.0 (C-1_{sp}), 52.4 (C-2_{sp}), 32.4 (m, C-6_{sp}), 32.0 (CH₂-5_{sp}), 29.80-28.4 (m, CH₂-5_{sp} x9 and CH₃-tBu-Boc), 27.4, 26.9 (CH₃-tBu-Si x2), 22.8 (C_q-tBu-Si), 22.6 (CH₂-5_{sp}), 20.0 (C_q-tBu-Si), 14.3 (C-18_{sp}); IR (neat): 3070, 2922, 2854, 1724, 1267, 1172, 1069, 827, 708 cm⁻¹; HRMS calculated for [C₅₃¹³C₅H₈₃NO₁₂Si + H]⁺: 1041.5748, found 1041.5748.

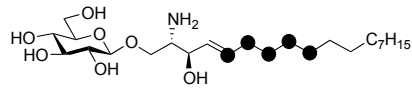
Glucosylsphingosine (37a). Protected glucosylsphingosine **36a** (130 mg, 0.128 mmol, 1.0 eq) was dissolved in



THF:pyridine (15 mL) and hydrogen fluoride (70% HF in pyridine) (53 μL, 0.256 mmol, 2.0 eq) was added. The reaction was stirred at room temperature until TLC showed full conversion (~ 2 hours) (*R*_{f(product)} = 0.75 (40% EtOAc in DCM)). The

reaction was concentrated *in vacuo*, re-dissolved in EtOAc (30 mL) and washed with 1 M HCl (30 mL), sat. aq. NaHCO₃ (30 mL), and brine (30 mL). The aqueous layers were extracted with EtOAc (30 mL) and the combined organics were dried (Na₂SO₄), filtered and concentrated *in vacuo*. The crude mixture was dissolved in MeOH (13 mL) and sodium methoxide (30% in methanol) (18 μL, 0.128 mmol, 1.0 eq) was added. The reaction was stirred over night at room temperature and the progress of the reaction was followed by HPLC-MS. Aqueous potassium hydroxide (0.5 M) (3.8 mL, 1.9 mmol, 15 eq) was added and the reaction was left stirring over night at room temperature. The reaction was then quenched with AcOH (0.73 mL, 13 mmol, 100 eq) and concentrated *in vacuo*. The crude reaction mixture was coevaporated in toluene and put on ice-bath before addition water (1 mL) and TFA (3 mL). The reaction was stirred for 2 minutes at 0 °C and was then diluted with toluene (20 mL) and concentrated *in vacuo*. Purification by HPLC-MS (52-62% B, following the general procedure for HPLC-MS purifications) produced the title compound (31 mg, 0.067 mmol, 53%) as a TFA adduct. [α]_D²²: -5.0 (c = 0.1 MeOH); ¹H NMR (600 MHz, MeOD-*d*₄) δ 5.87 (ddt, 1 H, *J* = 15.0, 6.8, 1.2 Hz, H-5_{sp}), 5.48 (ddt, 1 H, *J* = 15.4, 6.9, 1.5 Hz, H-4_{sp}), 4.33-4.29 (m, 2 H, H-1 and H-3_{sp}), 3.97-3.88 (m, 3 H, H-6 and H-1_{a-sp}), 3.66 (m, 1 H, H-1_{b-sp}), 3.40-3.32 (m, 2 H, H-5 and H-2_{sp}), 3.29-2.21 (m, 3 H, H-2, H-3 and H-4), 2.1 (q, 2 H, *J* = 7.2 Hz, H-6_{sp}), 1.42 (m, 2 H, H-7_{sp}), 1.36-1.22 (m, 20 H, H-8_{sp} to H-17_{sp}), 0.9 (t, 3 H, *J* = 7.0 Hz, H-18_{sp}); ¹³C NMR: (151 MHz, MeOD-*d*₄) δ 136.8 (C-5_{sp}), 128.4 (C-4_{sp}), 104.1 (C-1), 78.1 (C-4), 77.9 (C-5), 74.8 (C-3), 71.5 (C-2), 70.9 (C-3_{sp}), 67.3 (C-6), 62.5 (C-1_{sp}), 56.8 (C-2_{sp}), 33.4 (C-6_{sp}), 33.1, 30.82, 30.81 (2x), 30.78, 30.77, 30.66, 30.50, 30.41, 30.18, 23.6 (11x CH₂-5_{sp}), 14.2 (C-18_{sp}); IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm⁻¹; HRMS calculated for [C₂₄H₄₇NO₇ + H]⁺: 462.3431, found 462.3424.

Glucosyl-[5,6,7,8,9-¹³C₅]-Sphingosine (37b). Protected glucosyl-[5,6,7,8,9-¹³C₅]-Sphingosine **36a** (48 mg, 47 μmol,



1.0 eq) was dissolved in THF:pyridine (10 mL) and hydrogen fluoride (70% HF in pyridine) (20 μL, 94 μmol, 2.0 eq) was added. The reaction was stirred at room temperature until TLC

showed full conversion (~ 2 hours) (*R*_{f(product)} = 0.75 (40% EtOAc in DCM)). The reaction was concentrated *in vacuo*, re-dissolved in EtOAc (20 mL) and washed with 1 M HCl (20 mL), sat. aq. NaHCO₃ (20 mL), and brine (20 mL). The

aqueous layers were extracted with EtOAc (20 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude mixture was dissolved in MeOH (8 mL) and sodium methoxide (30% in methanol) (6.5 μL , 47 μmol , 1.0 eq) was added. The reaction was stirred over night at room temperature and the progress of the reaction was monitored by HPLC-MS. Aqueous potassium hydroxide (0.5 M) (1.4 mL, 0.7 mmol, 15 eq) was added and the reaction was left stirring over night at room temperature. The reaction was then quenched with AcOH (0.3 mL, 4.7 mmol, 100 eq) and concentrated *in vacuo*. The crude reaction mixture was coevaporated with toluene and put on an ice-bath before the addition of water (0.3 mL) and TFA (1 mL). The reaction was stirred for 2 minutes at 0°C and then diluted with toluene (20 mL) and was concentrated *in vacuo*. Purification by HPLC-MS (52–62% B, following the general procedure for HPLC-MS purifications) produced the title compound (10.7 mg, 23 μmol , 49%) as a TFA adduct. $[\alpha]_D^{22}$: -5.1 ($c = 0.1$ MeOH); ^1H NMR (600 MHz, $\text{MeOD}-d_4$) δ 5.85 (dm, 1 H, $J = 150.2$ Hz, H-5_{sp}), 5.48 (dt, 1 H, $J = 15.8$, 6.4 Hz, H-4_{sp}), 4.34–4.29 (m, 2 H, H-1 and H-3_{sp}), 3.97–3.88 (m, 3 H, H-6 and H-1_{a-sp}), 3.66 (dd, 1 H, $J = 11.7$, 6.1 Hz, H-1_{b-sp}), 3.40–3.31 (m, 2 H, H-5 and H-2_{sp}), 3.29–2.21 (m 3 H, H-2, H-3 and H-4), 2.10 (dm, 2 H, $J = 126.9$ Hz, H-6_{sp}), 1.56–1.15 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.90 (t, 3 H, $J = 7.0$ Hz, H-18_{sp}); ^{13}C NMR (151 MHz, $\text{MeOD}-d_4$) δ 136.8 (d, $J = 43.0$ Hz, C-5_{sp}), 128.2 (dd, $J = 72.5$, 3.5 Hz, C-4_{sp}), 104.1 (C-1), 78.1 (C-4), 77.9 (C-5), 74.9 (C-3), 71.5 (C-2), 70.9 (m, C-3_{sp}), 67.3 (C-6), 62.5 (C-1_{sp}), 56.8 (d, $J = 3.4$ Hz, C-2_{sp}), 33.6–32.9 (m, C-6_{sp} and CH₂-_{sp}), 30.9–29.6 (m, CH₂-_{sp} x10), 23.4 (CH₂-_{sp}), 14.5 (C-18_{sp}); IR (neat): 3300, 2918, 2851, 1670, 1433, 1200, 1134, 1074, 1024, 800, 721 cm^{-1} ; HRMS calculated for $[\text{C}_{19}^{13}\text{C}_5\text{H}_{47}\text{NO}_7 + \text{H}]^+$: 467.3598, found 467.3591.

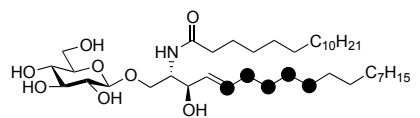
Glucosylceramide (38a). See general procedure for the synthesis of the ceramides from the sphingosine. Yield

(3.1 mg, 4.4 μmol , 57%); $R_f = 0.25$ (CHCl_3 :MeOH 9:1); $[\alpha]_D^{22}$: +6.0 ($c = 0.1$ MeOH:CHCl₃ 1:1); ^1H NMR (600 MHz, $\text{CDCl}_3/\text{MeOD}-d_4$) δ 5.68 (dt, 1 H, $J = 13.8$, 6.9 Hz, H-5_{sp}), 5.44 (dd, 1 H, $J = 15.3$, 7.8 Hz, H-4_{sp}), 4.26 (d, 1 H, $J = 7.9$ Hz, H-1'), 4.16 (dd, 1 H, $J = 10.3$, 4.8 Hz, H-1_{b-sp}), 4.06 (t, 1 H, $J = 8.4$ Hz, H-3_{sp}), 3.97 (dt, 1 H, $J = 8.4$, 4.0 Hz, H-2_{sp}), 3.86 (dd, 1 H, $J = 11.9$, 1.8 Hz, H-6_{a'}), 3.66 (m 1 H, H-6_{b'}), 3.59 (dd, 1 H, $J = 10.1$, 3.3 Hz, H-1_{a-sp}), 3.36 (m, 1 H, H-3'), 3.29–3.26 (m, 2 H, H-4' and H-5'), 3.21 (dd, 1 H, $J = 9.4$, 7.8 Hz, H-2'), 2.17 (t, 2 H, $J = 7.2$ Hz, H-2*), 2.02 (m, 2 H, H-6_{sp}), 1.58 (m, 2 H, H-3*), 1.42–1.23 (m, 46 H, H-7_{sp} to H-17_{sp} and H-4* to H-15*), 0.90 (t, 6 H, $J = 7.0$ Hz, H-18_{sp} and H-16*), ^{13}C NMR (151 MHz, $\text{CDCl}_3/\text{MeOD}-d_4$) δ 173.9 (C=O*), 133.0 (C-5_{sp}), 129.2 (C-4_{sp}), 102.5 (C-1'), 75.8 (C-4'), 75.7 (C-3'), 73.0 (C-2'), 70.9 (C-3_{sp}), 69.5 (C-5'), 67.8 (C-1_{sp}), 60.5 (C-6'), 52.6 (C-2_{sp}), 35.3 (C-2*), 31.4 (C-6_{sp}), 31.0, 28.80, 28.77 x3, 28.76 x4, 28.74 x2, 28.73, 28.71, 28.70, 28.64, 28.57, 28.50, 28.43, 28.42, 28.38, 28.37, 28.34, 25.10, 21.67 (CH₂ x24, C-7_{sp} to C-17_{sp} and C-3* to C-15*), 14.4 x2 (C-18_{sp} and C-16*); IR (neat): 3300, 2916, 2848, 1670, 1540, 1467, 1200, 1134, 1074, 1028, 721 cm^{-1} ; HRMS calculated for $[\text{C}_{40}\text{H}_{77}\text{NO}_8 + \text{H}]^+$: 700.5727, found 700.5720.

Glucosyl-2-N-([3,4,5- $^{13}\text{C}_3$]-hexadecanoyl)-sphingosine (38b) See general procedure for the synthesis of the

ceramides from the sphingosine. Yield (3.2 mg, 4.5 μmol , 59%); $R_f = 0.25$ (CHCl_3 :MeOH 9:1); $[\alpha]_D^{22}$: +4.8 ($c = 0.2$ MeOH:CHCl₃ 1:1); ^1H NMR (600 MHz, $\text{MeOD}-d_4$) δ 5.68 (dt, 1 H, $J = 13.8$, 6.9 Hz, H-5_{sp}), 5.44 (dd, 1 H, $J = 15.3$, 7.8 Hz, H-4_{sp}), 4.26 (d, 1 H, $J = 7.9$ Hz, H-1'), 4.16 (dd, 1 H, $J = 10.3$, 4.8 Hz, H-1_{b-sp}), 4.06 (t, 1 H, $J = 8.4$ Hz, H-3_{sp}), 3.97 (dt, 1 H, $J = 8.4$, 4.0 Hz, H-2_{sp}), 3.86 (dd, 1 H, $J = 11.9$, 1.8 Hz, H-6_{a'}), 3.66 (m 1 H, H-6_{b'}), 3.59 (dd, 1 H, $J = 10.1$, 3.3 Hz, H-1_{a-sp}), 3.36 (m, 1 H, H-3'), 3.29–3.26 (m, 2 H, H-4' and H-5'), 3.21 (dd, 1 H, $J = 9.4$, 7.8 Hz, H-2'), 2.17 (m, 2 H, H-2*), 2.02 (m, 2 H, H-6_{sp}), 1.58 (dm, 2 H, $J = 130$ Hz, H-3*), 1.42–1.16 (m, 46 H, H-7_{sp} to H-17_{sp} and H-4* to H-15*), 0.90 (t, 6 H, $J = 7.0$ Hz, H-18_{sp} and H-16*); ^{13}C NMR (151 MHz, $\text{MeOD}-d_4$) δ 176.0 (C=O*), 135.1 (C-5_{sp}), 131.3 (C-4_{sp}), 104.7 (C-1'), 78.0 (C-4'), 77.9 (C-3'), 75.2 (C-2'), 73.0 (C-3_{sp}), 71.6 (C-5'), 69.9 (C-1_{sp}), 62.6 (C-6'), 54.7 (C-2_{sp}), 34.8 (d, $J = 35.0$ Hz, C-2*), 33.1 (C-6_{sp}), 31.0–30.0 (m), 27.51, 27.4–27.0 (m), 26.87, 23.77 (CH₂ x24, C-7_{sp} to C-17_{sp} and C-3* to C-15*), 14.4 x2 (C-18_{sp} and C-16*); IR (neat): 3260, 2914, 2847, 1643, 1541, 1468, 1205, 1134, 1076, 1030, 717 cm^{-1} ; HRMS calculated for $[\text{C}_{37}^{13}\text{C}_3\text{H}_{77}\text{NO}_8 + \text{H}]^+$: 703.5828, found 703.5821.

Glucosyl-2-*N*-(hexadecanoyl)-[5,6,7,8,9-¹³C₅]-sphingosine (38c). See general procedure for the synthesis of the



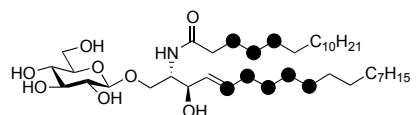
ceramides from the sphingosine. Yield (2.8 mg, 3.9 μ mol, 52%).

R_f = 0.25 (CHCl₃:MeOH 9:1); $[\alpha]_D^{22}$: +5.4 (c = 0.1 MeOH:CHCl₃);

¹H NMR (600 MHz, MeOD-*d*₄) δ 5.68 (dm, 1 H, J = 154.0 Hz, H-5_{sp}), 5.44 (m, 1 H, H-4_{sp}), 4.26 (d, 1 H, J = 7.9 Hz, H-1'), 4.16 (dd,

1 H, J = 10.3, 4.8 Hz, H-1_{b-sp}), 4.06 (m, 1 H, H-3_{sp}), 3.97 (m, 1 H, H-2_{sp}), 3.86 (dd, 1 H, J = 11.9, 1.8 Hz, H-6_a'), 3.66 (m, 1 H, H-6_b'), 3.59 (dd, 1 H, J = 10.1, 3.3 Hz, H-1_{a-sp}), 3.36 (m, 1 H, H-3'), 3.29-3.26 (m, 2 H, H-4' and H-5'), 3.21 (dd, 1 H, J = 9.4, 7.8 Hz, H-2'), 2.17 (m, 2 H, H-2*), 2.02 (dm, 2 H, J = 128.0 Hz, H-6_{sp}), 1.58 (dm, 2 H, J = 130.0 Hz, H-3*), 1.42-1.14 (m, 46 H, H-7_{sp} to H-17_{sp} and H-4* to H-15*), 0.90 (t, 6 H, J = 7.0 Hz, H-18_{sp} and H-16*); ¹³C NMR (151 MHz, MeOD-*d*₄) δ 176.0 (C=O*), 135.1 (d, J = 44.0 Hz, C-5_{sp}), 131.3 (d, J = 72.5 Hz, C-4_{sp}), 104.6 (C-1'), 78.0 (C-4'), 77.9 (C-3'), 75.2 (C-2'), 73.0 (C-3_{sp}), 71.6 (C-5'), 69.9 (C-1_{sp}), 62.6 (C-6'), 54.7 (C-2_{sp}), 37.4 (C-2*), 33.9-33.0 (m), 31.1-30.1 (m), 27.20, 23.78 (CH₂ x24, C-7_{sp} to C-17_{sp} and C-3* to C-15*), 14.5 (C-18_{sp} and C-16*); IR (neat): 3300, 2913, 2847, 1643, 1544, 1468, 1260, 1085, 1030, 718 cm⁻¹; HRMS calculated for [C₃₅¹³C₅H₇₇NO₈ + H⁺]: 705.5895, found 705.5906.

Glucosyl-2-*N*-([3,4,5-¹³C₃]-hexadecanoyl)-[5,6,7,8,9-¹³C₅]-sphingosine (38d). See general procedure for the



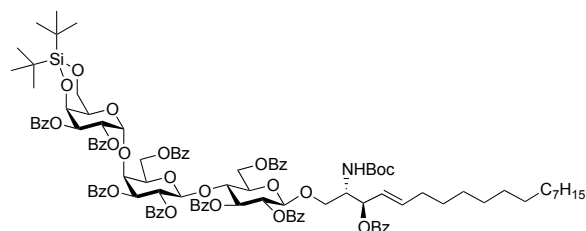
synthesis of the ceramides from the sphingosine. Yield (4.9 mg,

6.9 μ mol, 61%); R_f = 0.25 (CHCl₃:MeOH 9:1); $[\alpha]_D^{22}$: +5.0 (c = 0.2

MeOH:CHCl₃ 1:1); ¹H NMR (600 MHz, MeOD-*d*₄) δ 5.68 (dm, 1 H, J = 154.0 Hz, H-5_{sp}), 5.44 (m, 1 H, H-4_{sp}), 4.26 (d, 1 H, J = 7.9

Hz, H-1'), 4.16 (dd, 1 H, J = 10.3, 4.8 Hz, H-1_{b-sp}), 4.06 (m, 1 H, H-3_{sp}), 3.97 (m, 1 H, H-2_{sp}), 3.86 (dd, 1 H, J = 11.9, 1.8 Hz, H-6_a'), 3.66 (m, 1 H, H-6_b'), 3.59 (dd, 1 H, J = 10.1, 3.3 Hz, H-1_{a-sp}), 3.36 (m, 1 H, H-3'), 3.29-3.26 (m, 2 H, H-4' and H-5'), 3.21 (dd, 1 H, J = 9.4, 7.8 Hz, H-2'), 2.17 (m, 2 H, H-2*), 2.02 (dm, 2 H, J = 128.0 Hz, H-6_{sp}), 1.58 (dm, 2 H, J = 130.0 Hz, H-3*), 1.42-1.14 (m, 46 H, H-7_{sp} to H-17_{sp} and H-4* to H-15*), 0.90 (t, 6 H, J = 7.0 Hz, H-18_{sp} and H-16*); ¹³C NMR (151 MHz, MeOD-*d*₄) δ 176.0 (C=O*), 135.1 (d, J = 44.0 Hz, C-5_{sp}), 131.3 (d, J = 72.5 Hz, C-4_{sp}), 104.6 (C-1'), 78.0 (C-4'), 77.9 (C-3'), 75.2 (C-2'), 73.0 (C-3_{sp}), 71.6 (C-5'), 69.9 (C-1_{sp}), 62.6 (C-6'), 54.7 (C-2_{sp}), 37.4 (d, J = 35.0 Hz, C-2*), 33.9-33.0 (m), 31.1-30.0 (m), 27.50, 27.4-27.0 (m), 26.87, 23.78 (CH₂ x24, C-7_{sp} to C-17_{sp} and C-3* to C-15*), 14.4 (C-18_{sp} and C-16*); IR (neat): 3295, 2913, 2847, 1643, 1545, 1468, 1260, 1086, 1032, 718 cm⁻¹; HRMS calculated for [C₃₂¹³C₈H₇₇NO₈ + H⁺]: 708.5995, found 708.5989.

Globotriaosyl sphingosine (40a). Globotriaosyl imidate donor **39** (0.54 g, 0.33 mmol, 1.2 eq) and sphingosine



acceptor **23a** (0.14 g, 0.27 mmol, 1.0 eq)

were co-evaporated twice with toluene (5

mL) and then dissolved in anhydrous DCM

(3 mL). Activated mol sieves (3 Å) were

added and the mixture was stirred for one

hour at room temperature and then cooled

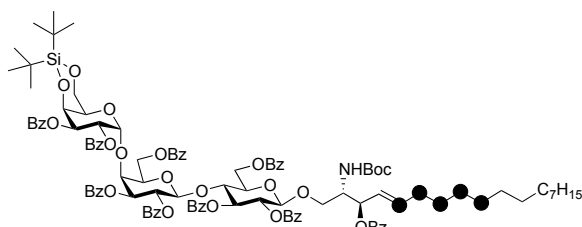
to 0 °C, before addition of BF₃·OEt₂ (48% in

Et₂O) (38 μ L, 0.3 mmol, 1.1 eq). The

reaction was stirred until TLC showed complete conversion of the sphingosine acceptor (~2 h). The reaction mixture was then transferred to an extraction funnel with EtOAc (40 mL) and washed with sat. aq. NaHCO₃ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na₂SO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (12% Et₂O, 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% Et₂O, 20% DCM in petroleum ether); $[\alpha]_D^{22}$: +31 (c = 1.0 CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.19 (m, 2 H, H_{arom}), 7.92 (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 (m, 2 H, 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 Hz, 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 NH_{Boc}), 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-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-sp}), 3.53 (m, 1 H, H-5'), 1.88 (m, 2 H, H-6_{sp}), 1.33 (s, 9 H, CH₃-tBu-Boc), 1.30-1.11 (m, 22 H, H-7_{sp} to H-17_{sp}), 1.06 (s, 9 H, CH₃-tBu-Si), 1.00 (s, 9 H, CH₃-tBu-Si), 0.87 (t, 3 H, $J = 6.8$ Hz, H-18_{sp}); ¹³C NMR (101 MHz, CDCl₃) 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-arom}), 130.1, 130.0 (CH_{arom} x2), 129.9 (C_{q-arom}), 129.8, 129.64, 129.59, 129.58, 129.5 (CH_{arom} x5), 129.4 (C_{q-arom}), 129.3 (CH_{arom}), 129.2, 129.0, 128.6, 128.50, 128.47 (C_{q-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-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, 29.3, 29.2, 28.7 (CH_{2-sp} x10), 28.2 (CH₃-tBu-Boc), 27.5, 27.2 (CH₃-tBu-Si x2), 23.2 (C_q-tBu-Boc), 22.6 (CH_{2-sp}), 20.7 (C_q-tBu-Si), 14.1 (C-18_{sp}); IR (neat): 3070, 2926, 2856, 1722, 1451, 1267, 1095, 1070, 1028, 708 cm⁻¹; HRMS calculated for [C₁₁₂H₁₂₇NO₂₈Si + Na]⁺: 1984.8206, found 1984.8204.

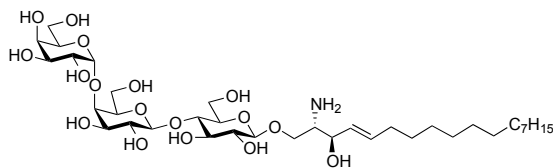
[5,6,7,8,9-¹³C₅]-Globotriaosyl sphingosine (40b). Globotriaosyl imidate donor (**39**) (158 mg, 96 μ mol, 1.2 eq)



and ¹³C₅-sphingosine acceptor **23b** (40.7 mg, 80 μ mol, 1.0 eq) were co-evaporated twice with toluene (5 mL) and then dissolved in anhydrous DCM (2 mL). Activated molsieves (3 Å) were added and the mixture was stirred at room temperature for 1 hour and then cooled to 0 °C, before addition of BF₃·OEt₂ (48% in Et₂O) (23 μ L, 88 μ mol, 1.1 eq). The reaction

was stirred until TLC showed complete conversion of the ¹³C₅-sphingosine acceptor (~2 h). The reaction mixture was then transferred to an extraction funnel with EtOAc (40 mL) and washed with sat. aq. NaHCO₃ (40 mL) and brine (30 mL). The aqueous layers were then extracted with EtOAc (40 mL) and the combined organics were dried (Na₂SO₄), filtered, and concentrated *in vacuo*. Purification by column chromatography (12% ether, 10% DCM in petroleum ether) **40b** as an amorphous solid (87 mg, 44 μ mol, 55%). $R_f = 0.54$ (30% ether, 20% DCM in petroleum ether); [α]_D²²: +30 ($c = 1.0$ CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.17 (m, 2 H, H_{arom}), 8.06 – 8.00 (m, 4 H, H_{arom}), 7.95 (m, 2 H, H_{arom}), 7.92 – 7.84 (m, 6 H, H_{arom}), 7.67 (m, 2 H, 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 (m, 2 H, H_{arom}), 7.11 (m, 2 H, 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-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-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 CH₃-tBu-Boc), 1.05 (s, 9 H, CH₃-tBu-Si), 1.00 (s, 9 H, CH₃-tBu-Si), 0.87 (t, 3 H, $J = 6.8$ Hz, H-18_{sp}); ¹³C NMR (101 MHz, CDCl₃) 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-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-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-sp}), 29.8 – 28.1 (m, CH_{2-sp} x9 and CH₃-tBu-Boc), 27.5, 27.2 (CH₃-tBu-Si x2), 23.2 (C_q-tBu-Si), 22.7 (C_{sp}), 20.7 (C_q-tBu-Si), 14.1 (C-18_{sp}); IR (neat): 3070, 2925, 2853, 1718, 1452, 1266, 1094, 1069, 706 cm⁻¹; HRMS calculated for [C₁₀₇¹³C₅H₁₂₇O₂₈Si + Na]⁺: 1989.8374, found 1989.8370.

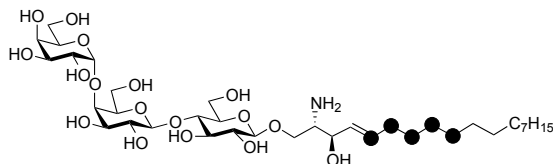
Globotriaosylsphingosine (41a). Protected globotriaosylsphingosine **40a** (200 mg, 0.10 mmol, 1.0 eq) was



dissolved in THF:pyridine 4:1 (20 mL) and hydrogen fluoride (70% HF in pyridine) (53 μ L, 0.26 mmol, ca. 20 eq) was added. The reaction was stirred at room temperature until TLC showed full conversion to a lower running spot (~4 h). 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 were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude mixture was then dissolved in methanol (20 mL) and sodium methoxide (30% in methanol) (14 μ L, 0.10 mmol, 1.0 eq) was added. The reaction was stirred over night at room temperature and the progression of the reaction was followed by HPLC-MS. Aqueous potassium hydroxide (0.5 M, 4.1 mL, 2.0 mmol, 20 eq) was added and the reaction left stirring over night at room temperature. The reaction was then quenched with AcOH (0.58 mL, 100 eq) and concentrated *in vacuo*. The crude reaction mixture was co-evaporated with toluene and put on an ice-bath before the addition of trifluoroacetic acid (5 mL). The reaction mixture was completely dissolved after one minute and was then stirred for another minute at 0 $^\circ\text{C}$. The solution was then transferred to a round bottom flask containing toluene (50 mL) and concentrated to about 10 mL *in vacuo*. The co-evaporation was repeated two times with toluene (40 mL), before concentration to dryness. The completion of the reaction was confirmed by HPLC-MS. The residue was then 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 ninhydrin spray). Purification by HPLC-MS (40-48% B, following the general procedure for HPLC-MS purifications) produced globotriaosylsphingosine **41a** (43 mg, 54 μ mol, 53%) as a TFA adduct. $[\alpha]_D^{22}$: +34.0 ($c = 0.5$ MeOH); ^1H NMR (600 MHz, $\text{MeOD}-d_4$) δ : 5.87 (m, 1 H, H-5_{sp}), 5.49 (m, 1 H, 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-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-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-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}), 1.36-1.22 (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, $\text{MeOD}-d_4$) 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 ($\text{CH}_2\text{-Sp}$ x11), 14.4 (C-18_{sp}); IR (neat): 3345 bs, 2925, 2855, 1674, 1202, 1134, 1067, 1027, 974, 801, 721 cm^{-1} ; HRMS calculated for $[\text{C}_{36}\text{H}_{67}\text{NO}_{17} + \text{H}]^+$: 786.4482, found 786.4485.

Globotriaosyl-[5,6,7,8,9- $^{13}\text{C}_5$]-sphingosine (41b). Globally protected globotriaosyl-[5,6,7,8,9- $^{13}\text{C}_5$]-sphingosine

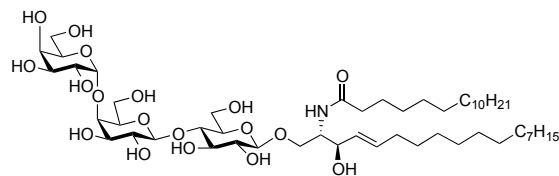


40b (87 mg, 0.45 μ mol, 1.0 eq) was dissolved in THF:pyridine 4:1 (10 mL) and hydrogen fluoride (70% HF in pyridine) (24 μ L, 0.11 mmol, ca. 20 eq) was added. The reaction mixture was stirred at room temperature until TLC showed full conversion to a lower running

spot (~4 h). The reaction was then concentrated *in vacuo*, re-dissolved in EtOAc (50 mL) and washed with 1N 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 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 μ L, 0.45 μ mol, 1.0 eq) was added. The reaction mixture was stirred over night at room 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 mixture was left stirring over night at room temperature. The reaction was then quenched with AcOH (0.25 mL, 100 eq) and concentrated *in vacuo*. The crude reaction mixture was co-evaporated with toluene and put on an ice-bath

before the addition of trifluoroacetic acid (3 mL). The product was completely dissolved in about 1 min and the reaction was stirred for an additional minute at 0 °C. The solution was then transferred to a round bottom flask containing toluene (50 mL) and concentrated *in vacuo* to about 10 mL. The co-evaporation was repeated twice with toluene (40 mL), before concentration to dryness. The completion of the reaction was monitored by HPLC–MS. The reaction mixture was filtered over a small silica column and eluted with MeOH/DCM 1:9 and H₂O/MeOH/DCM 3:27:70 (TLC visualised with ninhydrin spray). Purification by HPLC–MS (40–48% B, following the general procedure for HPLC–MS purifications) produced globotriaosylsphingosine **41b** (17 mg, 21 μmol, 48%) as a TFA adduct. $[\alpha]_D^{22}$: +33.0 (*c* = 0.20 MeOH); ¹H NMR (600 MHz, MeOD-*d*₄) δ 5.85 (dm, 1 H, *J* = 150.2 Hz, H-5_{sp}), 5.47 (m, 1 H, 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-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-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}); ¹³C 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-6''), 71.0 (C-4''), 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-sp}), 30.9 – 29.8 (m, CH_{2-sp} x10), 23.7 (CH_{2-sp}), 14.4 (C-18_{sp}); HRMS calculated for [C₃₁¹³C₅H₆₇NO₁₇H]⁺: 791.4650, found 791.4654.

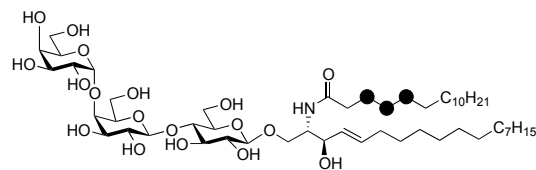
Globotriaosylceramide (42a). See general procedure for the synthesis of the ceramides from the sphingosine.



Yield (6 mg, 5.8 μg, 49%); *R*_f = 0.35 (CHCl₃:MeOH:H₂O 70:27:3); $[\alpha]_D^{22}$: +24 (*c* = 0.75 MeOH:CHCl₃ 1:1); ¹H NMR (600 MHz, CDCl₃/MeOD-*d*₄) δ 5.69 (dt, 1 H, *J* = 14.7, 6.9 Hz, H-5_{sp}), 5.45 (dd, 1 H, *J* = 15.3, 7.8 Hz, H-4_{sp}), 4.96 (d, 1 H, *J* = 3.8 Hz, H-1''), 4.41 (d, 1 H, *J* = 6.9 Hz, H-1'), 4.30 (d, 1 H, *J* = 7.8 Hz, H-1),

4.25 (ddd, 1 H, *J* = 6.8, 4.7, 1.3 Hz, H-3_{sp}), 4.19 (dd, 1 H, *J* = 10.1, 4.5 Hz, H-5''), 4.07 (t, 1 H, *J* = 8.8 Hz), 4.04–3.96 (m, 4 H), 3.92 (d, 1 H, *J* = 3.0 Hz) 3.89 (d, 1 H, *J* = 3.2 Hz), 3.85–3.81 (m, 3 H), 3.79–3.73 (m, 2 H), 3.71–3.3.63 (m, 3H), 3.60–3.51 (m, 4 H), 2.17 (t, 2 H, *J* = 7.2 Hz, H-2*), 2.03 (m, 2 H, H-6_{sp}), 1.58 (dm, 1 H, *J* = 130.0 Hz, H-3*), 1.43–1.20 (m, 46 H, H-7_{sp} to H-17_{sp} and H-4* to H-15'), 0.90 (t, 6 H, *J* = 6.9 Hz, H-18_{sp} and H-16*); ¹³C NMR (151 MHz, CDCl₃/MeOD-*d*₄) δ 177.5 (C=O*), 133.1 (C-5_{sp}), 132.6 (C-4_{sp}), 103.3 (C-1'), 102.3 (C-1), 100.6 (C-1''), 78.8 (C-4), 77.7 (C-4'), 74.3 (C-5' and C-5), 74.1 (C-2'), 72.8 (C-3), 72.5 (C-2), 70.9 (C-5''), 70.7 (C-3'), 70.5 (C-4''), 69.2 (C-3''), 68.9 (C-3_{sp}), 68.4 (C-2''), 67.8 (C-1_{sp}), 60.6 (C-6''), 58.8 (C-6), 57.2 (C-6'), 52.5 (C-2_{sp}), 35.3 (C-2*), 31.4 (C-6_{sp}), 31.0 28.78 x2, 28.77 x4, 28.74 x2, 28.72 x2, 28.71 x2, 28.64, 28.59, 28.54, 28.52, 28.44 x2, 28.41, 28.40, 28.38, 28.36, 21.7 (CH₂ x24, C-7_{sp} to C-17_{sp} and C-3* to C-15*), 12.5 (C-18_{sp} and C-16*); IR (neat): 3300, 2918, 2851, 1636, 1465, 1379, 1205, 144, 1070, 1016, 719 cm⁻¹; HRMS calculated for [C₅₂H₉₇NO₁₈ + H]⁺: 1024.6784, found 1024.6783.

Globotriaosyl-2-N-[[3,4,5-¹³C₃]-hexadecanoyl]-sphingosine (42b). See general procedure for the synthesis of the



ceramides from the sphingosine. Yield (9 mg, 8.7 μmol, 71%); *R*_f = 0.35 (CHCl₃:MeOH:H₂O 70:27:3); $[\alpha]_D^{22}$: +24 (*c* = 0.25 (MeOH:CHCl₃ 1:1); ¹H NMR (600 MHz, CDCl₃/MeOD-*d*₄) δ 5.69 (dt, 1 H, *J* = 14.7, 6.9 Hz, H-5_{sp}), 5.45 (dd, 1 H, *J* = 15.3, 7.8 Hz, H-4_{sp}), 4.96 (d, 1 H, *J* = 3.8 Hz, H-1''), 4.41

(d, 1 H, *J* = 6.9 Hz, H-1'), 4.30 (d, 1 H, *J* = 7.8 Hz, H-1), 4.25 (ddd, 1 H, *J* = 6.8, 4.7, 1.3 Hz, H-3_{sp}), 4.19 (dd, 1 H, *J* = 10.1, 4.5 Hz, H-5''), 4.07 (t, 1 H, *J* = 8.8 Hz), 4.04–3.96 (m, 4 H), 3.92 (d, 1 H, *J* = 3.0 Hz) 3.89 (d, 1 H, *J* = 3.2 Hz), 3.85–3.81 (m, 3 H), 3.79–3.73 (m, 2 H), 3.71–3.3.63 (m, 3H), 3.60–3.51 (m, 4 H), 2.17 (m, 2 H, H-2*), 2.03 (m, 2 H, H-6_{sp}), 1.58 (dm, 1 H, *J* = 130.0 Hz, H-3*), 1.43–1.14 (m, 46 H, H-7_{sp} to H-17_{sp} and H-4* to H-15'), 0.90 (t, 6 H, *J* = 6.9 Hz, H-18_{sp} and H-16*); ¹³C NMR (151 MHz, CDCl₃/MeOD-*d*₄) δ 133.0 (C-5_{sp}), 132.7 (C-4_{sp}), 100.7, 78.9, 74.8,

60.7, 52.6, 31.5, 31.3, 29.0-28.2 (m), 25.5-24.8 (m), 24.3-24.0 (m), 21.71, 21.60, 19.68 (CH₂ x24, C-7_{sp} to C-17_{sp} and C-3* to C-15*), 12.6 (C-18_{sp} and C-16*); IR (neat): 3300, 2914, 2849, 1632, 1551, 1470, 1370, 1203, 1070, 1024, 716 cm⁻¹; HRMS calculated for [C₄₉¹³C₃H₉₇NO₁₈ + H]⁺: 1027.6884, found 1027.6881.

Globotriaosyl-2-N-(hexadecanoyl)-[5,6,7,8,9-¹³C₅]-sphingosine (42c). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (5.5 mg, 5.3 μmol, 51%); R_f = 0.35 (CHCl₃:MeOH:H₂O 70:27:3); [α]_D²²: +26 (c = 0.15 MeOH:CHCl₃ 1:1); ¹H NMR (850 MHz, CDCl₃/MeOD-*d*₄) δ 5.69 (dm, 1 H, *J* = 150.0, H-5_{sp}), 5.45 (m, 1 H, H-4_{sp}), 4.96 (d, 1 H, *J* = 3.8

Hz, H-1''), 4.41 (d, 1 H, *J* = 6.9 Hz, H-1'), 4.30 (d, 1 H, *J* = 7.8 Hz, H-1), 4.25 (ddd, 1 H, *J* = 6.8, 4.7, 1.3 Hz, H-3_{sp}), 4.19 (dd, 1 H, *J* = 10.1, 4.5 Hz, H-5''), 4.07 (t, 1 H, *J* = 8.8 Hz), 4.04-3.96 (m, 4 H), 3.92 (d, 1 H, *J* = 3.0 Hz), 3.89 (d, 1 H, *J* = 3.2 Hz), 3.85-3.81 (m, 3 H), 3.79-3.73 (m, 2 H), 3.71-3.63 (m, 3H), 3.60-3.51 (m, 4 H), 2.17 (m, 2 H, H-2*), 2.03 (m, 2 H, H-6_{sp}), 1.58 (dm, 1 H, *J* = 130.0 Hz, H-3*), 1.43-1.14 (m, 46 H, H-7_{sp} to H-17_{sp} and H-4* to H-15*), 0.90 (t, 6 H, *J* = 6.9 Hz, H-18_{sp} and H-16*); ¹³C NMR (213 MHz, CDCl₃/MeOD-*d*₄) δ 174.0 (C=O*), 133.2 (d, *J* = 42.0 Hz, C-5_{sp}), 103.4 (C-1'), 102.4 (C-1), 100.7 (C-1''), 74.4, 72.9, 72.6, 70.9, 70.5, 69.0, 68.4, 67.6, 66.1, 60.8, 38.6 35.6, 31.8-31.0 (m), 29.1-28.1 (m), 25.2, 25.1, 21.8, 21.6, 19.7 (CH₂ x24, C-7_{sp} to C-17_{sp} and C-3* to C-15*), 12.6 (C-18_{sp} and C-16*); IR (neat): 3300, 2914, 2849, 1633, 1549, 1468, 1204, 1069, 1026, 719 cm⁻¹; HRMS calculated for [C₄₇¹³C₅H₉₇NO₁₈ + H]⁺: 1029.6951, found 1029.6949.

Globotriaosyl-2-N-([3,4,5-¹³C₃]-hexadecanoyl)-[5,6,7,8,9-¹³C₅]-sphingosine (42d). See general procedure for the

synthesis of the ceramides from the

sphingosine. Yield (8.6 mg, 8.3 μmol, 64%); R_f

= 0.35 (CHCl₃:MeOH:H₂O 70:27:3); [α]_D²²: +25

(c = 0.1 MeOH:CHCl₃ 1:1); ¹H NMR (850 MHz,

CDCl₃/MeOD-*d*₄) δ 5.69 (dm, 1 H, *J* = 150.0, H-

5_{sp}), 5.45 (m, 1 H, H-4_{sp}), 4.95 (d, 1 H, *J* = 3.8

Hz, H-1''), 4.41 (d, 1 H, *J* = 6.9 Hz, H-1'), 4.30

(d, 1 H, *J* = 7.8 Hz, H-1), 4.25 (m, 1 H, H-3_{sp}), 4.19 (dd, 1 H, *J* = 10.1, 4.5 Hz, H-5''), 4.07 (m, 1 H), 4.04-3.96 (m, 4 H), 3.92 (d, 1 H, *J* = 3.0 Hz), 3.89 (m, 1 H), 3.85-3.81 (m, 3 H), 3.79-3.73 (m, 2 H), 3.71-3.63 (m, 3H), 3.60-3.51 (m, 4 H), 2.17 (m, 2 H, H-2*), 2.02 (dm, 2 H, *J* = 128.0 Hz, H-6_{sp} H-6_{sp}), 1.65-1.14 (m, 48 H, H-7_{sp} to H-17_{sp} and H-3* to H-15'), 0.90 (t, 6 H, *J* = 6.9 Hz, H-18_{sp} and H-16*); ¹³C NMR (213 MHz, CDCl₃/MeOD-*d*₄) δ 174.0 (C=O*), 133.1 (d, *J* = 44.6 Hz, C-5_{sp}), 31.8-30.8 (m), 29.8-28.0 (m), 25.95, 25.27-24.95 (m), 21.67, 21.52, 19.56 (CH₂ x24, C-7_{sp} to C-17_{sp} and C-3* to C-15*), 12.4 (C-18_{sp} and C-16*); IR (neat): 3300, 2955, 2849, 1634, 1549, 1466, 1070, 1028, 719 cm⁻¹; HRMS calculated for [C₄₄¹³C₈H₉₇NO₁₈ + H]⁺: 1032.7052, found 1032.7053.

2.4 References and notes

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***N*-Fmoc-Protected Sphingosine is a Suitable Starting Material in the Synthesis of Glycosylsphingosines**

3.1 Introduction

In Chapter 2 the synthesis of carbon-13-labeled glucosylsphingosine **3** was described. Key step in this synthesis comprised glycosylation of *N*-Boc-protected sphingosine **1** using imidate **2** as the donor glucoside (Figure 3.1).^[1] During the research that led to the identification of 4,6-silylidene donor **2**, perbenzoylated glucopyranosyl imidates **4** and **5** were assessed as well. In these studies it was found that the use of stoichiometric $\text{BF}_3 \cdot \text{OEt}_2$ as the Lewis acid to activate these donors in the presence of *N*-Boc-sphingosine **1** did not

yield the desired, fully protected sphingosine **6**. Instead, removal of the *N*-Boc protective group was observed to occur as the main event. Arguably the lower reactivity of donors **4** and **5** when compared to donor **2** is behind this difference in glucosylation outcome.

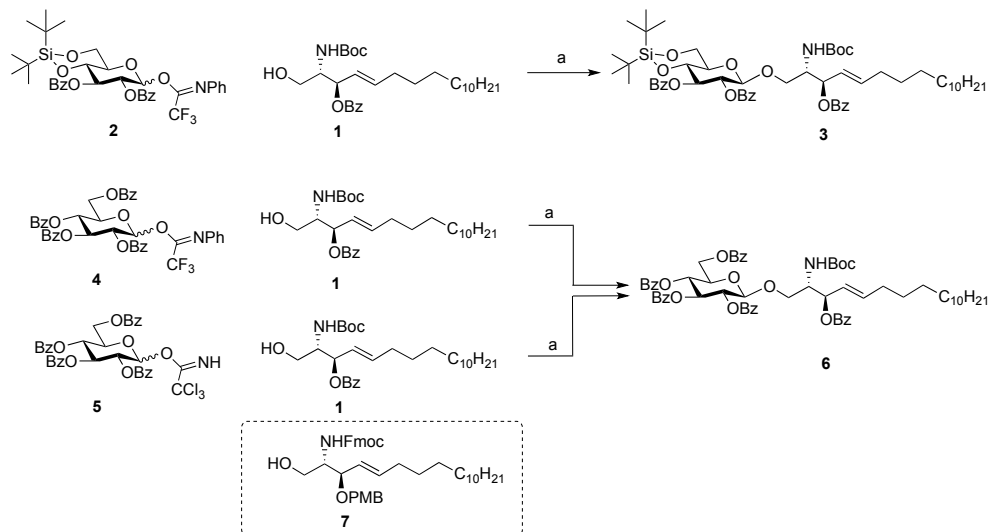


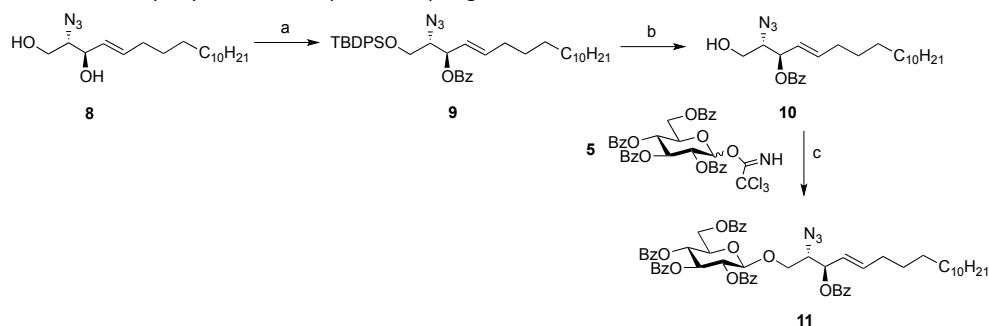
Figure 3.1. *N*-Boc-protected sphingosine **1** could be glucosylated with donor imidate **2**, but not with the comparatively less reactive donor glucosides **4** and **5**. Conditions: a) BF_3OEt_2 , DCM, 0 °C, 1 h, **3**: 49%; **6**: no productive yield from either **4** or **5**.

When looking more closely at the outcome in the glycosylation reaction of silylidene-protected glucosyl imidate **2** with *N*-Boc-protected sphingosine, the desired, fully protected glucosylsphingosine **3** was obtained in a rather moderate yield of 49%, and also this reaction was accompanied by partial loss of the *N*-Boc protective group. While the process proved effective enough to allow the synthesis of a panel of (carbon-13-labeled) glucosylsphingosine derivatives (see Chapter 2), there remained obvious room for improvement, especially when considering translation to functional analogues of compound **3**. With these observations in mind, attention was focused on the use of alternative amine protective group strategies, specifically on the nature of the protective groups by means of which the amine and the secondary alcohol of the sphingosine acceptor are temporarily blocked. As is described in this Chapter, glucosylation of sphingosine **7**, with the amine blocked with an Fmoc group and the secondary alcohol masked as the *para*-methoxybenzyl ether, proved to proceed more effectively when compared to the methodology described in Chapter 2. Moreover, besides glucosylation, also galactosylation could be accomplished, a transformation that could not be accomplished using acceptor **1**. This results allows the construction of galactosylsphingolipids in isotopically enriched form for lipidomics studies as well.

3.2 Results and discussion

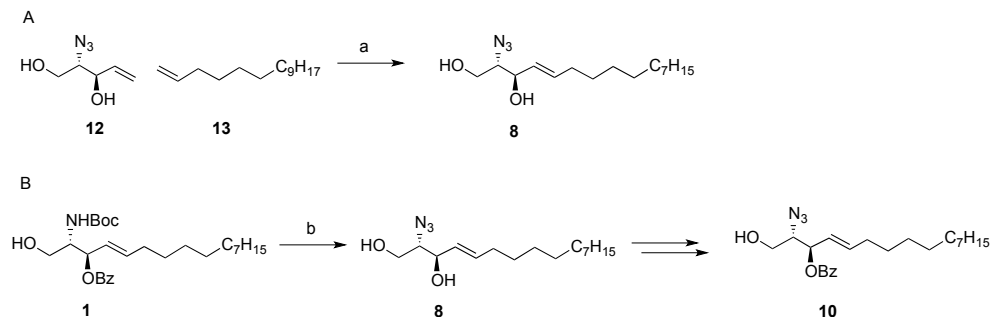
The efficacy of acceptor **10**,^[2,3] featuring protection of the amine in the sphingosine acceptor as the azide, in combination with the benzoyl group as protecting group for the secondary alcohol, in glycosylation events was investigated. As can be seen (Scheme 3.1), perbenzoylated glucosyl imidate **5** can be coupled to acceptor **10** (itself prepared in three steps from known azide **8**^[4]) under the agency of triflic acid to give **11** in good yield. Thus, the comparatively stronger protic acid (TfOH versus the Lewis acid BF_3OEt_2), which proved detrimental in the coupling of N-Boc-sphingosine **1** with donor **5**, could be used effectively in combination with sphingosine **10** (which does not bear any acid-labile protective groups).

Scheme 3.1. Glycosylation of azide-protected sphingosine.



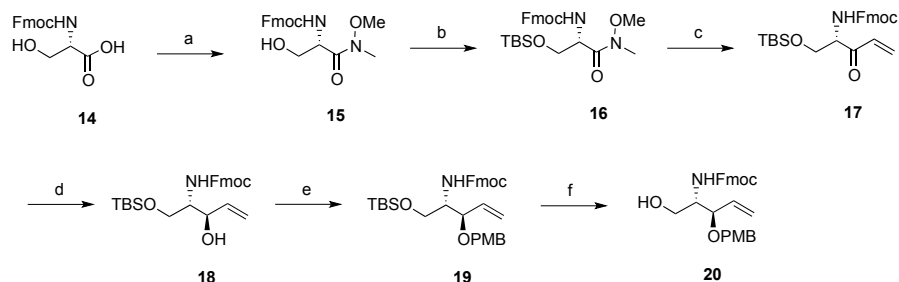
Reagents and conditions: (a) i) TBDPSCl, pyridine, 0 °C to r.t., 20 h; ii) BzCl, pyridine, r.t., 20 h, 91%; (b) TBAF, acetic acid, THF, 0 °C, 1 h, 62%. (c) TfOH, DCM, 0 °C, 1 h, 87%.

However and as was shown previously, the azide in terminal alkene **12** prevented its effective cross-metathesis with terminal alkene **13** (Scheme 3.2).^[5,6] Cross-metathesis of carbon-13-enriched alkene **13** to an appropriately protected form of alkene **12**, which itself can be prepared in an enantiomerically pure form from serine, is the strategy of choice for the preparation of neutron-encoded sphingosine bases, as was described in Chapter 2.^[1] On paper, one could produce $^{13}\text{C}_5$ -enriched, N-Boc protected sphingosine **1**, remove both O-benzoyl protection and N-Boc protection (in this order to avoid acyl migration from O to N), perform a diazo transfer, and then install the secondary benzoyl following the scheme as depicted for sphingosine **10**. Performing this, rather lengthy, protective group manipulation scheme on a (comparatively expensive) carbon-13-enriched compound however is suboptimal. Therefore, an alternative, partially protected sphingosine acceptor, that could be prepared through the cross-metathesis scheme, and that at the same time would be able to withstand rather acidic glycosylation conditions, was required. Such a sphingosine derivative was found in compound **7**, in which the amine is masked with an Fmoc group and the secondary alcohol as the *para*-methoxybenzyl ether.^[5,6]

Scheme 3.2. Synthesis of partially protected sphingosine **10**.

Reagents and conditions: (a) Grubbs 2nd generation catalyst, AcOH, CH₂Cl₂, reflux, 48 h; (b) NaOMe, MeOH, r.t., 20 h; (ii) KOH, H₂O, r.t., 20 h; (iii) TFA, H₂O, 0 °C, 30 min; (iv) imidazole-1-sulfonyl azide hydrochloride, K₂CO₃, CuSO₄·5 H₂O, MeOH, r.t., 20 h.

The synthesis of *N*-Fmoc protected sphingosine **7** was accomplished following the strategy published by Yamamoto and co-workers^[7] with as adaptation that, while the Yamamoto group started from Boc-L-serine, here Fmoc-L-serine **14** was employed as the starting material (Scheme 3.3). The carboxylic acid in **14** was converted to the Weinreb amide (EDC.HCl, *N,O*-dimethylhydroxylamine hydrochloride), followed by silylation of the primary hydroxyl with TBS-Cl, giving **16** in 75% yield over the two steps.

**Scheme 3.3** Synthesis of *N*-Fmoc-protected aminodiol

Reagents and conditions: (a) *N,O*-dimethylhydroxylamine hydrochloride, EDC.HCl, DIPEA, DCM, HOBT·H₂O, 0 °C to r.t., 20 h; (b) TBS-Cl, NMM, DMF, 0 °C to r.t., 20 h, 75% over two steps; (c) (i) vinylmagnesium bromide, THF, 0 °C, 1 h, 67%; (d) LiAlH(OtBu)₃, EtOH, -78 °C, 5 h, 73%; (e) PMB-*N*-phenyltrifluoroimidate, camphor-10-sulfonic acid, toluene, 0 °C to r.t., 20 h, 58%; (f) TBAF, acetic acid, THF, r.t., 20 h, 94%.

In the next step, Weinreb amide **16** was treated with vinylmagnesium bromide to give the α,β -unsaturated ketone **17**. The stable tetrahedral intermediate that is formed upon addition of the Grignard reagent and that collapses only during work-up (addition of 2M HCl) ensures that a second Grignard addition is prevented.^[8,9] Ketone **17** was then reduced in a stereoselective fashion to give allylic alcohol **18** using LiAlH(OtBu)₃ as reducing agent.^[7,10] The alcohol moiety in **18** was next protected as the *para*-methoxybenzyl ether

(PMB)^[11] after which the primary alcohol was unmasked (**19** to **20**), both steps using standard protective group manipulation conditions.

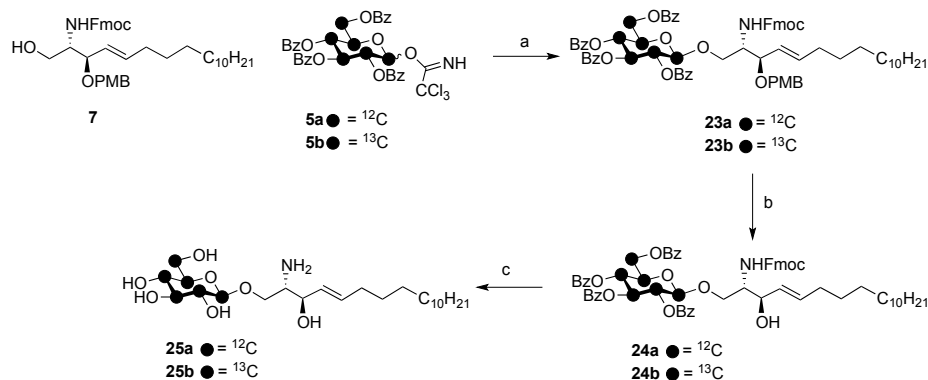
At this stage, three functionalized pentene derivatives were available for an ensuing cross-metathesis: partially protected alkenes **18** and **20** and fully protected alkene **19**. All three were subjected to a cross-metathesis reaction with 2 equivalents of linear, terminal alkene **13** and Grubbs' 2nd generation catalyst in the presence of acetic acid to prevent (see Chapter 2) alkene migration (Table 3.1).^[1,12] As can be seen, the partially protected alkenes **18** and **20** perform about equally well in this process, whereas the fully protected alkene **19** delivers the desired product in low yield only.

Table 3.1. Optimization of cross-metathesis mediated assembly of *N*-Fmoc-protected sphingosine.

Reaction scheme: Pentene derivatives **18**, **19**, **20** (where R₁O is on the left and NHFmoc and OR₂ are on the right) react with linear terminal alkene **13** (CH₂=CH-CH₂-CH₂-CH₂-C₁₀H₂₁) under the following conditions: Grubbs 2nd, AcOH, DCM, 40 °C, 2 days. The products are **21**, **22**, and **7**, where the pentene part is replaced by the C₁₀H₂₁ chain.

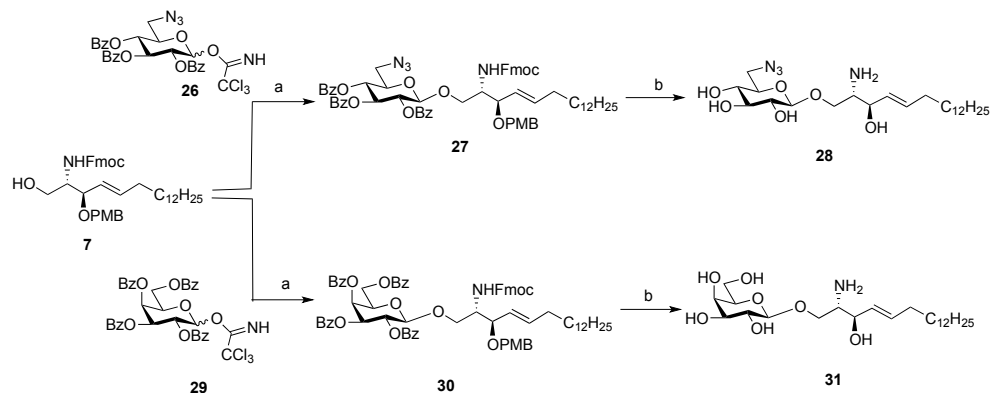
| entry | pentene | R ₁ | R ₂ | Product | Yield (%) |
|----------|-----------|----------------|----------------|-----------|-----------|
| 1 | 18 | TBS | H | 21 | 62 |
| 2 | 19 | TBS | PMB | 22 | 18 |
| 3 | 20 | H | PMB | 7 | 65 |

Both because of the (slightly) higher yield and because the partially protected sphingosine produced from **20** would allow modification of the primary alcohol directly, the process summarized in entry 3 is the most suitable for preparation of ¹³C₅-enriched sphingosine derivatives. For this to become feasible, the compatibility of Fmoc-sphingosine **7** in glycosylation events needed to be established. In a first attempt, glycosylation of **7** with imidate **5** under the agency of BF₃OEt₂ (Scheme 3.4) proved abortive. Switching to the stronger Lewis acid, TMSOTf (10 mol%) led to the formation of the desired, fully protected glycosylated sphingosine **23** in good yield, thus revealing that switching from *N*-Boc-protection to *N*-Fmoc-protection, as was hypothesized, pays off.^[5,13] Removal of the *para*-methoxybenzyl group (10% TFA in DCM), followed by global removal (sodium hydroxide in a mixture of methanol and methylene chloride) of the benzoyl and *N*-Fmoc protective groups yielded β-glucosylsphingosine **25** in 80% yield based on **23**. All spectroscopic and analytical data on **25** were in full agreement with the data obtained on the same compound as synthesized in Chapter 2.^[1] In a similar vein, but now starting from ¹³C₆-glucose donor **5b**, the corresponding isotopically enriched glucosylsphingosine **25b** could be prepared.

Scheme 3.4 Synthesis of $^{13}\text{C}_6$ -glucosylsphingosine **25a/b** using *N*-Fmoc-protected sphingosine **7**.

Reagent and conditions: (a) (i) 10 mol% TMSOTf, DCM -20 °C, 1 h, (82%); (b) 10% TFA in DCM, 0 °C, 3 h; (c) (i) NaOH, DCM/MeOH (3:1), r.t., 20 h; (ii) acetic acid, r.t., 80% over the two steps.

The usefulness of sphingosine **7** as acceptor in glycosphingolipid synthesis is finally demonstrated in the construction of 6-deoxy-6-azidoglucosylsphingosine **28** and galactosylsphingosine **31** (Scheme 3.5). As can be seen, TMSOTf-catalyzed glycosylation of **7** with either donor imidate **26** or **29** proceeded uneventfully and the same holds true for the global deprotection of the resulting glycosylsphingosines **27** and **30**, to produce compounds **28** and **31**, respectively.

Scheme 3.5 Synthesis of 6-azido-glucosylsphingosine **28** and galactosylsphingosine **31**.

Reagent and conditions: (a) (i) 10 mol% TMSOTf, DCM -20 °C, 1 h, (79% **27**, 74% **30**); (b) i) 10% TFA in DCM, 0 °C, 3 h; (ii) NaOH, DCM/MeOH (3:1), r.t., 20 h.; (iii) acetic acid, r.t., 75% **28**, 77% **31** over two steps.

3.3 Conclusion

In conclusion, this Chapter describes an improved strategy, hinging on the use of *N*-Fmoc-protected sphingosine **7** as acceptor in glycosylation events, for the synthesis of glycosphingolipids. The main advantages in using **7** instead of *N*-Boc sphingosine **1** (which

was employed in Chapter 2) are in improved yields, and in the potential use of strong Lewis acids. This in turn allows the synthesis of structural and functional glucosylsphingosine analogues, as is demonstrated by the construction of 6-deoxy-6-azidoglucosylsphingosine **28** (with an azide installed for bioconjugation purposes) and galactosylsphingosine **31**. The latter compound is thought to be the secondary storage material^[14-16] in Krabbe disease (deficiency in galactocerebrosidase)^[17] and the strategy presented here, in combination with the methodology developed in Chapter 2 for the construction of ¹³C-labeled sphingosine, allows for the construction of neutron-encoded galactosylsphingosine for quantitative lipidomics studies in the context of this lysosomal storage disorder.

3.4 Experimental section

General Remarks: Commercially available reagents and solvents (Acros, Fluka, or Merck) were used as received, unless otherwise stated. CH₂Cl₂ and THF were freshly distilled before use, over P₂O₅ and Na/benzophenone, respectively. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide. Traces of water were removed from starting compounds by co-evaporation with toluene. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out using forced flow of the indicated solvent systems on Screening Devices silica gel 60 (40–63 µm mesh). Size-exclusion chromatography was carried out on Sephadex LH20 (MeOH/CH₂Cl₂, 1:1). Analytical TLC was carried out on aluminum sheets (Merck, silica gel 60, F254). Compounds were visualized by UV absorption (254 nm), or by spraying with ammonium molybden/cerium sulphate solution [(NH₄)₆Mo₇O₂₄·4H₂O (25 g/L), (NH₄)₄Ce(SO₄)₆·2H₂O (10 g/L), 10 % sulphuric acid in ethanol] or phosphormolybdic acid in EtOH (150 g/L), followed by charring (ca. 150 °C). IR spectra were recorded with a Shimadzu FTIR-8300 instrument and are reported in cm⁻¹. Optical rotations were measured with a Propol automatic polarimeter (sodium D-line, λ = 589 nm). ¹H and ¹³C NMR spectra were recorded with a Bruker AV 400 MHz spectrometer at 400.2 (¹H) and 100.6 (¹³C) MHz, or with a Bruker AV 600 MHz spectrometer at 600.0 (¹H) and 151.1 (¹³C) MHz. Chemical shifts are reported as δ values (ppm), and were referenced to tetramethylsilane (δ = 0.00 ppm) directly in CDCl₃, or using the residual solvent peak (D₂O). Coupling constants (*J*) are given in Hz, and all ¹³C spectra were proton decoupled. NMR assignments were made using COSY and HSQC, and in some cases TOCSY experiments. LC-MS analysis was carried out with an LCQ Advantage Max (Thermo Finnigan) instrument equipped with a Gemini C18 column (Phenomenex, 50, 4.6 mm, 3 µm), using the following buffers: A: H₂O, B: acetonitrile, and C: aq. TFA (1.0 %).

General procedure for the glycosylation of *N*-Fmoc-protected sphingosine **7 and trichloro-imidate donors.**

Protected sphingosine (1 eq) and imidate-donor (1.5 eq) were co-evaporated twice with toluene before it was dissolved in dry DCM (0.1 M) under protected atmosphere. To the solution was added activated 4 Å molsieves and was stirred for 30 minute. The reaction mixture was cooled to -20 °C, followed by activation with TMSOTf (10% mol, based on sphingosine). The reaction was stirred at -20 °C until, according TLC, all sphingosine acceptor was consumed (approximately 1 hour). When the reaction was complete, the reaction mixture was neutralized with triethylamine and filtered over Celite. The reaction was concentrated *in vacuo* followed by purification by silica gel chromatography or with size exclusion column giving protected glycosylsphingosine.

General procedure for deprotection of protected glycosylsphingosine. The protected sphingosine (1 eq) was dissolved in DCM (0.1 M) and was cooled to 0 °C. To the solution was added TFA (10 volume%) and stirred for 4 hours allowing to reach room temperature. The mixture was diluted with toluene and concentrated *in vacuo*. The crude mixture was dissolved in DCM/MeOH (1:1, 0.1 M) and sodium hydroxide (6 eq) and stirred overnight

at room temperature. The reaction was according LC-MS finished and the reaction was neutralized with AcOH and concentrated *in vacuo*. The crude product was purified with silica column chromatography (10% MeOH in chloroform to 3:27:70 H₂O:MeOH:CHCl₃) giving fully deprotected sphingosine.

(9H-fluoren-9-yl)methyl (S)-(3-hydroxy-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate (15).

To a solution of DIPEA (8.2 mL, 46.9 mmol, 0.9 eq) in DCM (300 mL) was added Fmoc-L-Serine **14** (17.1 g, 52.2 mmol, 1 eq). The mixture was stirred under argon at 0 °C. 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, followed by addition of Weinreb salt (6.12 g, 62.6 mmol, 1.2 eq) and DIEA (4.5 mL, 26.1 mmol, 0.5 eq). The reaction was stirred over night, which was allowed to reach room temperature. The mixture was washed 2x 2M HCl, 2x sat. aq. NaHCO₃ and brine. All water layers were extracted with DCM and the combined organic layers were dried with MgSO₄, filtered and concentrated *in vacuo* giving resulting oil **10**, which was used in the next step without further purification. *R*_f = 0.39 (EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 7.76 (d, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 7.64–7.56 (m, 2 H, CH_{Fmoc}), 7.40 (t, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 7.31 (t, 2 H, CH_{Fmoc}), 5.92 (d, 1 H, *J* = 8.3 Hz, NH), 4.87 (m, 1 H, H-2), 4.40 (d, 2 H, *J* = 7.1 Hz, CH_{2-Fmoc}), 4.22 (t, 1 H, *J* = 7.0 Hz, CH_{Fmoc}), 3.86 (s, 2H, H-1), 3.78 (s, 3 H, CH_{3-OMe}), 3.25 (s, 3 H, CH_{3-NMe}), 2.52 (bs, 1 H, OH); ¹³C NMR (101 MHz, CDCl₃) δ 156.0 (C=O_{Fmoc}) 143.93, 143.82, 2x 141.39 (C_{q-Fmoc}), 141.46 (C-3), 127.84, 127.21, 125.25, 120.10 (4x CH_{Fmoc}), 67.35 (CH_{2-Fmoc}), 63.70 (C-1), 61.79 (CH_{3-OMe}), 52.88 (C-2), 47.26 (CH_{Fmoc}) 31.91 (CH_{3-OMe}); IR (neat): 3412, 3315, 2941, 1714, 1448, 1263, 1053 cm⁻¹; HRMS calculated for [C₂₀H₂₂N₂O₅ + H]⁺: 371.1537, found 371.1531.

(9H-fluoren-9-yl)methyl (S)-(3,8,8,9,9-pentamethyl-4-oxo-2,7-dioxa-3-aza-8-siladecan-5-yl)carbamate (16).

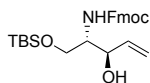
Crude product **15** was dissolved in dry DMF (500 mL) under protected atmosphere and cooled to 0 °C. To the solution was added TBDMS-Cl (10.2 g, 67.6 mmol, 1.3 eq) followed by addition of NNM (6.3 mL, 57.2 mmol, 1.1 eq). The mixture was stirred over night, which was allowed to reach room temperature. The reaction mixture was diluted with H₂O was added and was extracted twice with diethyl ether. 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 yellow oil **16** (18.9 g, 39.0 mmol, 75% over two steps). *R*_f = 0.95 (EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 7.76 (d, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 7.62 (t, 2 H, *J* = 8.2 Hz, CH_{Fmoc}), 7.40 (t, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 7.32 (t, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 5.72 (d, 1 H, *J* = 8.7 Hz, NH), 4.85 (m, 1 H, H-2), 4.37 (d, 2H, *J* = 7.2 Hz, CH_{2-Fmoc}), 4.25 (t, 1 H, *J* = 7.3 Hz, CH_{Fmoc}), 3.96–3.83 (m, 2 H, H-1), 3.77 (s, 3 H, CH_{3-OMe}), 3.25 (s, 3 H, CH_{3-NMe}), 0.90 (s, 9 H, TBS_{tBu}), 0.06 (s, 6 H, 2x TBS_{Me}); ¹³C NMR (101 MHz CDCl₃) δ 168.0 (C-3), 156.0 (C=O_{Fmoc}) 143.93, 143.82, 2x 141.39 (C_{q-Fmoc}), 127.82, 127.20, 125.34, 120.09, (4x CH_{Fmoc}) 67.27 (CH_{2-Fmoc}), 63.48 (C-1), 61.65 (CH_{3-OMe}), 53.16 (C-2), 47.28 (CH_{Fmoc}), 32.62 (CH_{3-NMe}), 25.93 (TBS_{tBu}), 18.26 (TBS_{tBu-q}), -5.35 (TBS_{Me}); IR (neat) 3305, 3057, 2924, 1712, 1450, 1053 cm⁻¹; HRMS calculated for [C₂₆H₃₆N₂O₅Si + H]⁺: 485.2401, found 485.2404.

(9H-fluoren-9-yl)methyl (S)-(1-((tert-butyldimethylsilyl)oxy)-3-oxopent-4-en-2-yl)carbamate (17).

Silylated protected **16** (4.8 g, 10 mmol, 1 eq) was in dry THF (20 mL) under protected atmosphere and cooled to 0 °C. To the solution was added slowly vinyl MgBr (1 M in THF, 40 mL, 40 mmol, 4 eq) and the reaction was stirred for one hour at 0 °C. The reaction mixture was quenched by slowly adding it to cold 2M HCl (200 mL) and quickly extracted twice with EtOAc. 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 **17** (3 g, 6.7 mmol, 67%). *R*_f = 0.75 (15% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, 2 H, *J* = 7.5 Hz, H_{Arom}), 7.62 (t, 2 H, *J* = 6.8 Hz, H_{Arom}), 7.41 (t, 2 H, *J* = 7.5 Hz, H_{Arom}), 7.32 (t, 2 H, *J* = 7.5, H_{Arom}), 6.57 (dd, 1 H, *J* = 17.2, 10.8 Hz, H-4), 6.39 (d, 1 H, H-5_a), 5.90–5.83 (m, 2 H, H-5_b + NH), 4.70 (m, 1 H, H-2), 4.39 (d, 2 H, *J* = 7.2 Hz, CH_{2-Fmoc}), 4.24 (t, 1 H, *J* = 7.2 Hz, CH_{Fmoc}), 4.05 (d, 1 H, *J* = 10.2 H-1_a), 3.90 (dd, 1 H, *J* = 10.4, 4.0 H-1_b), 0.86 (s, 9 H, TBS_{tBu}), 0.02 (s, 3 H, TBS_{Me}), 0.01 (s, 3 H, TBS_{Me}); ¹³C NMR (101 MHz CDCl₃) δ 196.9 (C-3) 155.96 (C=O_{Fmoc}), 144.05, 143.93, 2x 141.44 (C_{q-Fmoc}), 133.14 (C-4), 129.9 (C-5), 127.85, 127.21, 125.30, 120.12 (4x CH_{Fmoc}), 67.26 (CH_{2-Fmoc}), 63.47 (C-1), 60.05 (C-2), 47.31 (CH_{Fmoc}), 25.86 (TBS_{tBu}), 18.33 (TBS_{tBu-q}), -5.44 (TBS_{Me}); IR (neat) 2953, 2927, 2854, 1699, 1500, 1450,

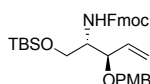
1251, 1026 cm^{-1} ; HRMS calculated for $[\text{C}_{26}\text{H}_{33}\text{N}_1\text{O}_4\text{Si} + \text{H}]^+$: 452.2799, found 452.2783.

(9H-fluoren-9-yl)methyl ((2S,3R)-1-((tert-butyldimethylsilyl)oxy)-3-hydroxypent-4-en-2-yl)carbamate (18).



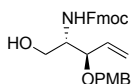
Allic ketone **17** (3.0 g, 6.7 mmol, 1 eq) was dissolved in EtOH (500 mL) under an protected atmosphere and cooled to -78°C . To the solution was added $\text{LiAlH}(\text{OtBu})_3$ (3.7 g, 14.7 mmol, 2.2 eq) and for 5 hours at -78°C and was quenched with 0.1 M HCl (175 mL) at the same temperature. The mixture was quickly extracted twice with EtOAc and the combined organic layers were washed with 0.1 M HCl and brine, dried with MgSO_4 , filtered and concentrated *in vacuo*. The crude oil was purified with silica gel chromatography (2.5-10% EtOAc in pentane) giving a white solid **18** (2.2 g, 4.9 mmol, 73%) and starting material (0.7 g, 1.6 mmol, 23 %). $R_f = 0.52$ (15% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.77 (d, 2 H, $J = 7.5$ Hz, H_{Fmoc}), 7.58 (m, 2 H, H_{Fmoc}), 7.40 (t, 2 H, $J = 7.5$ Hz, H_{Fmoc}), 7.31 (m, 2 H, H_{Fmoc}), 5.94 (m, 1 H, H-4), 5.56 (d, 1 H, $J = 8.6$ Hz, NH), 5.41 (d, 1 H, $J = 17.1$ Hz, H-5_a), 5.27 (d, 1 H, $J = 10.6$ Hz, H-5_b), 4.39 (d, 2 H, $J = 7.2$ Hz, $\text{CH}_2\text{-Fmoc}$), 4.31 (m, 1 H, H-3), 4.24 (t, 1 H, $J = 7.0$ Hz, CH_{Fmoc}), 3.97 (dd, 1 H, $J = 10.4$, 2.8 Hz, H-1_a), 3.78 (d, 1 H, $J = 10.2$, 3.2 Hz, H-1_b), 3.37 (m, 1 H, H-2), 3.38 (d, 1 H, $J = 8.4$ Hz, OH), 0.91 (s, 9 H, TBS_{tBu}), 0.07 (s, 6 H, 2x TBS_{Me}); ^{13}C NMR (101 MHz CDCl_3) δ 156.3 (C=O_{Fmoc}), 144.06, 143.97, 2x 141.44 (4x $\text{C}_{\text{q-Fmoc}}$), 137.34 (C-4), 127.83, 127.18, 125.22, 120.14 (4x CH_{Fmoc}), 116.36 (C-5), 74.88 (C-3), 66.95 ($\text{CH}_2\text{-Fmoc}$), 62.35 (C-1), 55.19 (C-2), 47.34 (CH_{Fmoc}), 25.94 (TBS_{tBu}), 18.24 ($\text{TBS}_{\text{tBu-q}}$), -5.50 (TBS_{Me}); IR (neat): 3439, 2927, 2854, 1705, 1251, 1080 cm^{-1} ; HRMS calculated for $[\text{C}_{26}\text{H}_{35}\text{NO}_4\text{Si} + \text{H}]^+$: 453.2343, found 453.2348.

(9H-fluoren-9-yl)methyl ((2S,3R)-1-((tert-butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)oxy)pent-4-en-2-yl)carbamate (19).



Allylic alcohol **18** was dissolved in dry DCM toluene (1 mL) under protected atmosphere, followed by addition of activated 4 Å molsieves and PMB-fluoro imidate (0.11 g, 0.36 mmol, 1.5 eq) The mixture was stirred for 30 minutes, after which was cooled to 0°C before addition of 10-Camphorsulphonic acid (7 g, 0.024 mmol, 0.1 eq). The mixture was stirred over night and was allowed reaching room temperature. The mixture was diluted with DCM and filtered over Celite, washed with sat. aq. NaHCO_3 (aq) and brine. The water layers were extracted with DCM and the combined organic layers were dried with MgSO_4 , filtered and concentrated *in vacuo*. The product was purified with silica gel chromatography (2.5-10 % EtOAc in pentane) giving a slightly yellow solid **19** (0.08 g, 0.13 mmol, 58%). $R_f = 0.5$ (10% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.75 (d, 2 H, $J = 7.5$ Hz, H_{Fmoc}), 7.57 (m, 2 H, H_{Fmoc}), 7.39 (t, 2 H, $J = 7.5$ Hz, H_{Fmoc}), 7.29 (t, 2 H, $J = 7.5$ Hz, H_{Fmoc}), 7.23 (d, 2 H, $J = 7.5$ Hz, H_{PMB}), 6.86 (d, 2 H, $J = 8.0$ Hz, H_{PMB}), 5.83 (m, 1 H, H-4), 5.52 (d, 1 H, $J = 8.4$, NH), 5.42-5.34 (m, 2 H, H-5_a and NH), 5.06 (d, 1 H, $J = 9.7$ Hz, H-5_b), 4.54 (d, 1 H, $J = 11.3$ Hz, $\text{CH}_2\text{-PMB-a}$), 4.34-4.12 (m, 5 H, $\text{CH}_2\text{-Fmoc}$, CH_{Fmoc} , $\text{CH}_2\text{-PMB-b}$ and H-3), 3.96 – 3.87 (m, 2 H, H-1_a and H-2), 3.78 (s, 3 H, OMe_{PMB}), 3.65 (dd, 1H, $J = 10.0$, 4.1 Hz, H-1_b), 0.90 (d, 9 H, $J = 2.9$ Hz, TBS_{tBu}), 0.06 (s, 3 H, TBS_{Me}), 0.05 (s, 3 H, TBS_{Me}); ^{13}C NMR (101 MHz, CDCl_3) δ 156.32 (C=O_{Fmoc}), 144.02, 141.37 (4x $\text{C}_{\text{q-Fmoc}}$), 136.02 (C-4), 130.3 (CH_{PMB}), 129.4, 127.74, 127.48, 125.20, 120.12 (4x $\text{C}_{\text{q-Fmoc}}$), 116.33 (C-5), 113.8 (CH_{PMB}), 74.81 (C-3), 70.50 ($\text{CH}_2\text{-PMB}$), 66.98 ($\text{CH}_2\text{-Fmoc}$), 62.24 (C-1), 55.39 (OMe_{PMB}), 55.13 (C-2), 47.30 (CH_{Fmoc}), 25.92 (TBS_{tBu}), 18.22 ($\text{TBS}_{\text{tBu-q}}$), -5.50 (TBS_{Me}); IR (neat): 3444, 3317, 3070, 2950, 2854, , 1242 cm^{-1} ; HRMS calculated for $[\text{C}_{34}\text{H}_{43}\text{NO}_5\text{Si} + \text{H}]^+$: 573.2919, found 573.2923.

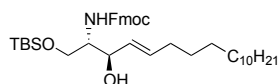
(9H-fluoren-9-yl)methyl ((2S,3R)-1-hydroxy-3-((4-methoxybenzyl)oxy)pent-4-en-2-yl)carbamate (20).



solution of **19** (0.25 g, 0.43 mmol, 1 eq) in 2 mL THF was added acetic acid (0.1 mL, 1.7 mmol, 4 eq) followed addition of TBAF (1 M in THF, 0.9 mL, 0.86 mmol, 2 eq). The reaction mixture was stirred over night at room temperature. The reaction was diluted with EtOAc, washed with sat. NaHCO_3 (aq), 1 M HCl (aq), and brine. The water layers were extracted with EtOAc and the combined organic layers were dried with MgSO_4 , filtered and concentrated *in vacuo*. The crude mixture was purified with silica gel chromatography (10-40% EtOAc in pentane) giving a white solid **20** (0.19 g, 0.4 mmol, 94 %). $R_f = 0.05$ (20% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.76 (d, 2 H, $J = 7.6$ Hz, H_{Fmoc}), 7.63 – 7.55 (m, 2H, H_{Fmoc}), 7.40 (t, 2 H, $J = 7.8$ Hz, H_{Fmoc}), 7.31 (t, 2 H, $J = 7.2$ Hz, H_{Fmoc}), 7.22 (d, 2 H, $J = 8.3$ Hz, H_{PMB}), 6.86 (d, 2 H, $J = 7.9$ Hz, H_{PMB}), 5.82 (m, 1-H, H-4), 5.52 (d, 1 H, $J = 8.5$ Hz, H-5_a), 5.44 – 5.35 (m, 2 H, H-5_b + NH), 4.59 (d, 1 H, $J = 11.3$ Hz, $\text{CH}_2\text{-PMB-a}$), 4.36 (d, 2 H, $J = \text{CH}_2\text{-Fmoc}$), 4.27-4.18 (m, 2 H, CH_{Fmoc} and $\text{CH}_2\text{-PMB-b}$), 4.10 (m, 1 H, H-3), 4.01 (d, 1

H, $J = 11.6$ Hz, H-1_b), 3.76 (s, 3 H, OMe_{PMB}), 3.68 (m, 1 H, H-2), 3.63 (dd, 1 H, $J = 11.2$, 2.4 Hz, H-1_b); ^{13}C NMR (101 MHz, CDCl_3) δ 156.31 (C=O_{Fmoc}), 144.06, 141.41 (4x C_{q-Fmoc}), 136.09 (C-4), 129.92 (CH_{PMB}), 127.83, 127.19, 125.23 (C-4), 125.29, 120.08 (4x CH_{Fmoc}), 116.37, (C-5), 114.03 (CH_{PMB}), 74.89 (C-3), 70.46 (CH_{2-PMB}), 66.96 (CH_{2-Fmoc}), 62.20 (C-1), 55.36 (OMe_{PMB}), 55.31 (C-2), 47.30 (CH_{Fmoc}); IR (neat): 3487, 3340, 3050, 2880, 1674, 1250 cm^{-1} ; HRMS calculated for $[\text{C}_{28}\text{H}_{29}\text{NO}_5 + \text{H}]^+$: 460.2054, found 460.2059.

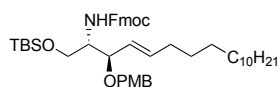
(9H-fluoren-9-yl)methyl ((2S,3R,E)-1-(tert-butyldimethylsilyloxy)-3-hydroxy-octadec-4-en-2-yl)carbamate (21).



To a solution of **18** (1.1 g, 2.45 mmol, 1 eq) in DCM (15 mL), 1-pentadecene (2.12 mL, 4.9 mmol, 2 eq) was added in a 500 mL RBF and stirred. Acetic acid (0.14 mL, 2 mmol, 1 eq) and Grubbs 2nd Generation (0.21 g, 0.25 mmol, 0.1 eq) were added and stirred for 2 days in a closed system at 40 °C. The resulting

mixture was evaporated and separated using column chromatography on silica gel (10-40% EtOAc in pentane) giving a brownish solid (0.91 mg, 1.47 mmol 62%). $R_f = 0.15$ (10% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.76 (d, 2 H, $J = 7.6$ Hz, H_{Fmoc}), 7.60 (m, 2 H, H_{Fmoc}), 7.40 (t, 2 H, $J = 7.2$ Hz, H_{Fmoc}), 7.31 (t, 2 H, $J = 7.6$ Hz, H_{Fmoc}), 5.78 (m, 1 H, H-5), 5.52 (m, 2 H, H-4 and NH), 4.38 (d, 2 H, $J = 7.2$ Hz, CH_{2-Fmoc}), 4.24 (m, 2 H, H-3 and CH_{Fmoc}), 3.98 (d, 1 H, $J = 10.0$ Hz, H-1_b), 3.78 (d, 1 H, $J = 10.0$ Hz, H-1_b), 3.67 (m, 1 H, H-2), 3.26 (d, 1 H, $J = 8.0$ Hz, OH), 2.05 (m, 2 H, H-6), 1.43 (m, 2 H, H-7), 1.37-1.19 (m, 20 H, H-8 to H-17), 0.91 (s, 9 H, TBS_{tBu}), 0.87 (t, 3 H, $J = 6.8$ Hz, H-18), 0.08 (s, 6 H, TBS_{Me}); ^{13}C NMR (101 MHz, CDCl_3) δ 156.31 (C=O_{Fmoc}), 144.11, 144.01, 141.44 (x2) (4x C_{q-Fmoc}), 133.57 (C-5), 129.32 (C-4), 127.82, 127.17, 125.22, 124.90, 120.11 (CH_{Fmoc}), 74.64 (C-3), 67.16 (CH_{2-Fmoc}), 63.53 (C-1), 54.97 (C-2), 47.34 (CH_{Fmoc}), 32.44, 32.06, 29.83, 29.80, 29.77, 29.65, 29.50, 29.35, 29.33 (11x CH₂ C-6 to C-17), 25.95 (TBS_{tBu}), 22.83 (CH₂ C-6 to C-17), 18.26 (TBS_{tBu-q}), -5.47 (TBS_{Me}); IR (neat): 3441, 2924, 2852, 1716, 1463, 1263, 1089 cm^{-1} ; HRMS calculated for $[\text{C}_{39}\text{H}_{61}\text{NO}_4\text{Si} + \text{H}]^+$: 636.4450, found 636.4452.

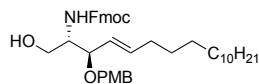
(9H-fluoren-9-yl)methyl ((2S,3R,E)-1-(tert-butyldimethylsilyloxy)-3-((4-methoxybenzyl)oxy)octadec-4-en-2-yl)carbamate (22).



Fully protected sphingosine **19** (50 mg, 0.09 mmol, 1 eq) was dissolved in dry DCM (0.5 mL), followed by addition of 1-pentadecene (47 μL , 0.17 mmol, 2 eq). To the reaction was added acetic acid (5 μL , 0.09 mmol, 1 eq) and catalytic amount of Grubbs 2nd catalyst (7 mg, 0.009 mmol, 0.1 eq).

The reaction mixture was stirred at 40 °C in closed vessel for 2 days. The resulting mixture was concentrated and purified by silica column chromatography (5% EtOAc in pentane) giving a brownish solid **22** (12 mg, 0.016 mmol 18%). $R_f = 0.75$ (10% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.75 (d, 2 H, $J = 7.6$ Hz, H_{Fmoc}), 7.57 (d, 2 H, $J = 7.2$ Hz, H_{Fmoc}), 7.38 (t, 2 H, $J = 7.2$ Hz, H_{Fmoc}), 7.29 (t, 2 H, $J = 7.6$ Hz, H_{Fmoc}), 7.21 (d, 2 H, $J = 7.2$ Hz, H_{PMB}), 6.88 (d, 2 H, $J = 6.8$ Hz, H_{PMB}), 5.71 (dt, 1 H, $J = 12.4$, 6.4 Hz, H-5), 5.44 (dd, 1 H, $J = 15.6$, 8.4 Hz, H-4), 5.06 (d, 1 H, $J = 9.2$ Hz, NH), 4.53 (d, 1 H, $J = 11.2$ Hz, CH_{2-PMB-a}), 4.30 (m, 2 H, CH_{2-Fmoc}), 4.24-4.19 (m, 2 H, CH_{Fmoc} and CH_{2-PMB-b}), 3.92 (dd, 1 H, $J = 10.0$, 3.2 Hz, H-1_a), 3.86 (m, 1 H, H-3), 3.82-3.74 (m, 4 H, OMe_{PMB} and H-2), 3.67 (dd, 1 H, $J = 10.0$, 4.0 Hz, H-1_b), 2.05 (m, 2 H, H-6), 1.34 (m, 2 H, H-7), 1.30-1.20 (m, 20 H, H-7 to H-17), 0.90 (s, 9 H, TBS_{tBu}), 0.88 (t, 3 H, $J = 6.8$ Hz, H-18), 0.06 (s, 3 H, TBS_{Me}), 0.05 (s, 3 H, TBS_{Me}); ^{13}C NMR (101 MHz, CDCl_3) δ 156.04 (C=O_{Fmoc}), 144.21, 144.05, 141.38, 141.36 (4x C_{q-Fmoc}), 136.98 (C-5), 130.63 (C_{q-PMB}), 129.84, 127.73 (2x CH_{Fmoc}), 127.57 (C-4), 127.08, 125.26 (2x CH_{Fmoc}), 120.05, 113.75 (2x CH_{PMB}), 79.09 (C-3), 70.00 (CH_{2-PMB}), 66.92 (CH_{2-Fmoc}), 61.64 (C-1), 55.54 (C-2), 55.34 (OMe_{PMB}), 47.31 (CH_{Fmoc}), 32.46 (C-6), 32.05, 30.40, 29.81, 29.78, 29.61, 29.49, 29.37, 29.34, 28.01, (10x CH₂ C-7 to C-17), 25.97 (TBS_{tBu}), 18.38 (TBS_{tBu-q}), 14.26 (C-18), -5.27 (TBS_{Me}), -5.35 (TBS_{Me}); IR (neat): 3444, 2924, 2852, 1726, 1450, 1264, 1082, 1056, 1037 cm^{-1} ; HRMS calculated for $[\text{C}_{47}\text{H}_{69}\text{NO}_5\text{Si} + \text{H}]^+$: 765.5025, found 765.5023.

(9H-fluoren-9-yl)methyl ((2S,3R,E)-1-hydroxy-3-((4-methoxybenzyl)oxy)octadec-4-en-2-yl)carbamate (7).

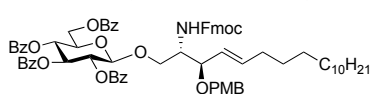


Fmoc-protected sphingosine **20** (1.95 g, 4.25 mmol, 1 eq) was dissolved in 20 mL DCM under protected atmosphere. 1-Pentadecene (2.3 mL, 8.5 mmol, 2 eq) and acetic acid (0.24 mL, 4.25 mmol, 1 eq) were added before addition of Grubbs 2nd catalyst (370 mg, 0.4 mmol, 0.1 eq). The mixture was stirred for 2 days at 40 °C.

The reaction mixture was concentrated *in vacuo* and the crude residue was purified with silica gel chromatography (0-15% EtOAc in pentane) giving a white solid **7** (1.77 g, 2.76 mmol, 65%). $R_f = 0.31$ (30% EtOAc

in pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.76 (d, 2 H, J = 7.7 Hz, H_{Fmoc}), 7.63 – 7.55 (m, 2 H, H_{Fmoc}), 7.40 (t, 2 H, J = 8.2 Hz, H_{Fmoc}), 7.31 (t, 2 H, J = 8.4 Hz, H_{Fmoc}), 7.20 (md, 2 H, J = 8.4 Hz, H_{PMB}), 6.84 (d, 2 H, J = 8.4 Hz, H_{PMB}), 5.78 (m, 1 H, H-5), 5.55 (d, 1 H, J = 8.3 Hz, N-H), 5.46 (d, 1 H, J = 15.4, 6.5, Hz, H-4), 4.56 (d, 1 H, J = 11.4 Hz, $\text{CH}_2\text{-PMB-a}$), 4.34 (m, 2 H, $\text{CH}_2\text{-Fmoc}$), 4.24-4.18 (m, 2 H, CH_{Fmoc} and $\text{CH}_2\text{-PMB-b}$), 4.07-3.97 (m, 2 H, H-1_a and H-3), 3.74 (s, 3 H, OMe_{PMB}), 3.71 – 3.60 (m, 2 H, H-1_b and H-2), 2.08 (m, 2H, H6), 1.26-1.14 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, J = 6.7 Hz, H-18); ^{13}C NMR (101 MHz, CDCl_3) δ 159.5 (C=O_{Fmoc}), 144.08, 141.45 (4x $\text{C}_{\text{q-Fmoc}}$), 137.16 (C-5), 129.88 (CH_{PMB}), 127.83, 127.19 (2x CH_{Fmoc}), 125.23 (C-4), 125.29, 120.08 (2x CH_{Fmoc}), 114.03 (CH_{PMB}), 81.61 (C-3), 70.44 ($\text{CH}_2\text{-PMB}$), 66.95 ($\text{CH}_2\text{-Fmoc}$), 62.19 (C-1), 55.36 (OMe_{PMB}), 55.35 (C-2), 47.35 (CH_{Fmoc}), 32.46 (C-6), 32.05, 29.83, 29.81, 29.79, 29.76, 29.70, 29.58, 29.49, 29.43, 29.32, 29.22, 22.82 (11x CH_2 , C-7 to C-17), 14.26 (C-18); IR (neat): 3464 and 3325, 2924, 2854, 1689, 1249, 1233 cm^{-1} ; HRMS calculated for $[\text{C}_{41}\text{H}_{55}\text{NO}_5 + \text{H}]^+$: 642.4160, found 642.4157.

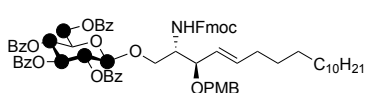
Glucosylsphingosine (23a). See general procedure for the glycosylation of protected sphingosine **7** with



trichloro-imidate donor **5a**. Yield (0.1 mg 0.082 mmol, 82%). R_f = 0.4 (20% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 8.05 – 7.70 (m, 10 H, H_{arom}), 7.56 – 7.16 (m, 20 H, H_{arom}), 6.81 (d, 2 H, J = 8.6 Hz, H_{PMB}), 5.93 (t, 1 H, J = 10.0 Hz, H-3'), 5.71 (t, 1 H, J = 10.0 Hz, H-4'),

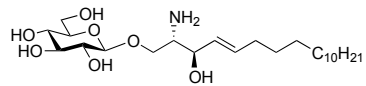
5.54 (t, 1 H, J = 8.0 Hz, H-2'), 5.45 (m, 1 H, H-5_{sp}), 5.29 (m, 1 H, H-4_{sp}), 4.89 (d, 1 H, J = 8.8 Hz, NH_{sp}), 4.78 (d, 1 H, J = 8.0 Hz, H-1'), 4.64 (dd, 1 H, J = 8.4, 2.8 Hz, $\text{CH}_2\text{-PMB-a}$), 4.52 (m, 1 H, $\text{CH}_2\text{-PMB-b}$), 4.43 (d, 1 H, J = 11.2 Hz, $\text{CH}_2\text{-Fmoc-a}$), 4.36-4.24, (m, 3 H, H-1_{sp-a}, $\text{CH}_2\text{-Fmoc-b}$ and H-6'a), 4.15-4.04 (m, 2 H, H-5' and H-6'b), 3.83 (m, 1 H, H-2_{sp}), 3.75-3.72 (m, 4 H, H-3_{sp} and OMe_{PMB}), 3.69 (m, 1 H, H-1_{sp-b}), 1.92 (m, 2 H, H-6_{sp}), 1.35-1.19 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.88 (t, 3 H, J = 6.8 Hz, H-18_{sp}); ^{13}C NMR (101 MHz, CDCl_3) δ 166.3, 165.9, 165.4, 165.3 (4x C=O_{Bz}), 155.89 (C=O_{Fmoc}), 144.15, 144.00, 141.42, 141.36 (4x $\text{C}_{\text{q-Fmoc}}$), 137.36 (C-5_{sp}), 133.61, 133.45, 133.40, 133.25 (4x CH_{arom}), 130.47 ($\text{C}_{\text{q-arom}}$), 129.96, 129.94, 129.88, 129.85, 129.64, 129.61, 129.50, 129.11, 128.88, 128.57, 128.57, 128.50, 128.45, 127.81, 127.79, 127.16 (CH_{arom} and $\text{C}_{\text{q-arom}}$), 125.23 (C-4_{sp}), 120.09 (CH_{Fmoc}), 113.84 (CH_{PMB}), 101.69 (C-1'), 79.08 (C-3_{sp}), 72.82 (C-3'), 72.37 (C-2' and C-5'), 70.32 ($\text{CH}_2\text{-PMB}$), 69.81 (C-4'), 68.55 (C-1_{sp}), 66.67 (C-6'), 63.28 ($\text{CH}_2\text{-Fmoc}$), 55.33 (OMe_{PMB}), 53.86 (C-2_{sp}), 47.33 (CH_{Fmoc}), 32.05 (C-6_{sp}), 29.84, 29.81, 29.79, 29.58, 29.49, 29.33, 29.28, 22.82, 22.82 (11x CH_2 , C-7_{sp} to C-17_{sp}), 14.27 (C-18_{sp}); IR (neat): 2925, 2856, 1721, 1690, 1250, 1231, 1089, 1066, 1025 cm^{-1} ; HRMS calculated for $[\text{C}_{75}\text{H}_{81}\text{NO}_{14} + \text{H}]^+$: 1220.5665, found 1220.5669.

[1,2,3,4,5,6- $^{13}\text{C}_6$]-Glucosylsphingosine (23b). See general procedure for the glycosylation of protected



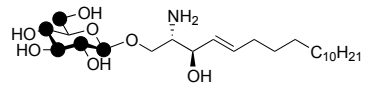
sphingosine **7** with trichloro-imidate donor **5b**. Yield (51 mg 0.041 mmol, 82%). R_f = 0.4 (20% EtOAc in pentane); ^1H NMR (^{13}C -decoupled, 400 MHz, CDCl_3) δ 8.05 – 7.80 (m, 10 H, H_{arom}), 7.59 – 7.22 (m, 20 H, H_{arom}), 6.84 (d, 2 H, J = 8.4 Hz, H_{PMB}), 5.93 (m, 1 H, H-3'), 5.71 (m, 1 H, H-4'), 5.54 (m, 1 H, H-2'), 5.45 (m, 1 H, H-5_{sp}), 5.29 (m, 1 H, H-4_{sp}), 4.93 (d, 1 H, J = 8.8 Hz, NH_{sp}), 4.78 (m, 1 H, H-1'), 4.64 (m, 1 H, $\text{CH}_2\text{-PMB-a}$), 4.52 (m, 1 H, $\text{CH}_2\text{-PMB-b}$), 4.48 (d, 1 H, J = 11.2 Hz, $\text{CH}_2\text{-Fmoc-a}$), 4.36-4.28, (m, 3 H, H-1_{sp-a}, $\text{CH}_2\text{-Fmoc-b}$ and H-6'a), 4.13-4.08 (m, 2 H, H-5' and H-6'b), 3.83 (m, 1 H, H-2_{sp}), 3.75-3.72 (m, 4 H, H-3_{sp} and OMe_{PMB}), 3.69 (m, 1 H, H-1_{sp-b}), 1.92 (m, 2 H, H-6_{sp}), 1.35-1.19 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.88 (t, 3 H, J = 6.8 Hz, H-18_{sp}); ^{13}C NMR (101 MHz, CDCl_3) δ 166.12, 165.84, 165.36, 165.16 (4x C=O_{Bz}), 155.87 (C=O_{Fmoc}), 144.03, 143.86, 141.34, 141.15 (4x $\text{C}_{\text{q-Fmoc}}$), 137.04 (C-5_{sp}), 133.59, 133.44, 133.38, 133.23 (4x CH_{arom}), 130.44, 129.93, 129.86, 129.83, 129.58, 129.48, 128.90, 128.86, 128.64, 128.55, 128.48, 128.43, 127.80, 127.77, 127.14 (CH_{arom} and $\text{C}_{\text{q-arom}}$), 125.21 (C-4_{sp}), 120.07 (CH_{Fmoc}), 113.82 (CH_{PMB}), 101.40 (d, J = 41.0 Hz, C-1), 78.94 (C-3_{sp}), 74.22-71.83 (m, C-2' and C-5'), 70.82-68.38 (m, C-4', $\text{CH}_2\text{-PMB}$, C-1_{sp}), 63.4 (d, J = 45 Hz, C-6'), 60.51 ($\text{CH}_2\text{-Fmoc}$), 55.31 (OMe_{PMB}), 54.34 (C-2_{sp}), 47.30 ($\text{CH}_2\text{-Fmoc}$), 32.03 (C-6_{sp}), 29.79, 29.77, 29.57, 29.47, 29.31, 29.26, 22.80, 21.16 (C-7_{sp} to C-17_{sp}), 14.30 (C-18_{sp}); IR (neat): 2925, 2856, 1721, 1690, 1250, 1231, 1089, 1066, 1025 cm^{-1} ; HRMS calculated for $[\text{C}_{69}\text{-}^{13}\text{C}_6\text{H}_{81}\text{NO}_{14} + \text{H}]^+$: 1226.5239, found 1226.5252.

Glucosylsphingosine (25a). General procedure for deprotection of protected glycosylsphingosine. Yield (30 mg,

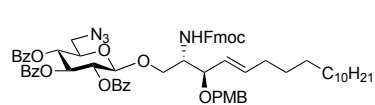


0.06 mmol, 80%). $[\alpha]_D^{22}$: -5.0 ($c = 0.1$ MeOH); ^1H NMR (600 MHz, MeOD- d_4) δ 5.87 (dtd, 1 H, $J = 15.0, 6.8, 1.2$ Hz, H-5_{sp}), 5.48 (ddt, 1 H, $J = 15.4, 6.9, 1.5$ Hz, H-4_{sp}), 4.33-4.29 (m, 2 H, H-1 and H-3_{sp}), 3.97-3.88 (m, 3 H, H-6 and H-1_{a-sp}), 3.66 (m, 1 H, H-1_{b-sp}), 3.40-3.32 (m, 2 H, H-5 and H-2_{sp}), 3.29-2.21 (m, 3 H, H-2, H-3 and H-4), 2.1 (q, 2 H, $J = 7.2$ Hz, H-6_{sp}), 1.42 (m, 2 H, H-7_{sp}), 1.36-1.22 (m, 20 H, H-8_{sp} to H-17_{sp}), 0.9 (t, 3 H, $J = 7.0$ Hz, H-18_{sp}); ^{13}C NMR: (151 MHz, MeD- d_4) δ 136.8 (C-5_{sp}), 128.4 (C-4_{sp}), 104.1 (C-1), 78.1 (C-4), 77.9 (C-5), 74.8 (C-3), 71.5 (C-2), 70.9 (C-3_{sp}), 67.3 (C-6), 62.5 (C-1_{sp}), 56.8 (C-2_{sp}), 33.4 (C-6_{sp}), 33.1, 30.82, 30.81 (2x), 30.78, 30.77, 30.66, 30.50, 30.41, 30.18, 23.6 (11x CH_{2-sp}), 14.2 (C-18_{sp}); IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm⁻¹; HRMS calculated for [C₂₄H₄₇NO₇ + H]⁺: 462.3431, found 462.3424.

[1,2,3,4,5,6- $^{13}\text{C}_6$]-Glucosylsphingosine (25b). General procedure for deprotection of protected glycosylsphingosine. Yield (15 mg, 0.03 mmol, 78%). $[\alpha]_D^{22}$: -4.8 ($c = 0.1$ MeOH); ^1H NMR (^{13}C -decoupled, 400 MHz, MeOD- d_4) δ 5.87 (m, 1 H, H-5_{sp}), 5.52 (m, 1 H, H-4_{sp}), 4.31 (m, 2 H, H-1 and H-3_{sp}), 4.05-3.82 (m, 3 H, H-6, H-1_{a-sp}), 3.69 (m, 1 H, H-1_{b-sp}), 3.56-3.39 (m, 2 H, H-5 and H-2_{sp}), 3.31-3.10 (m, 3 H, H-2, H-3, H-4), 2.10 (m, 2 H, H-6_{sp}), 1.42 (m, 2 H, H-7_{sp}), 1.38-1.22 (m, 20 H, H-8_{sp} to H-17_{sp}), 0.88 (m, 3 H, H-18_{sp}); ^{13}C NMR (101 MHz, MeOD- d_4) δ 131.4 (C-5_{sp}), 128.7 (C-4_{sp}), 104.1 (d, $J = 46.0$ Hz, C-1), 78.4-76.04 (m, C-4 and C-5), 74.7 (dd, $J = 52.0, 45.0$ Hz, C-3), 71.5 (m, C-2 and C-3_{sp}), 67.3 (d, $J = 43.0$ Hz, C-6), 62.5 (C-1_{sp}), 56.8 (C-2_{sp}), 33.0 (C-6_{sp}), 30.75, 30.72 (3x), 30.78, 30.77, 30.60, 30.50, 30.43, 30.36, 23.7 (11x CH_{2-sp}), 14.4 (C-18_{sp}); IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm⁻¹; IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm⁻¹; HRMS calculated for [C₁₈ $^{13}\text{C}_6$ H₄₇NO₇ + H]⁺: 468.3005, found 468.2996.

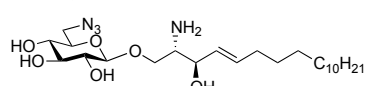


6-deoxy-6-Azido-Glucosylsphingosine (27). See general procedure for the glycosylation of protected sphingosine



7 with trichloro-imidate donor **26**. Yield (0.18 g, 0.16 mmol, 79%) $R_f = 0.55$ (20% EtOAc in pentane); ^1H NMR (400 MHz, CDCl₃) δ 7.95-7.89 (m, 4 H, H_{arom}), 7.85-7.76 (m, 4 H, H_{arom}), 7.55-7.50 (m, 2 H, H_{arom}), 7.45 (m, 15 H, H_{arom}), 6.87 (d, 2 H, $J = 8.8$ Hz, H_{PMB}), 5.90 (t, 1 H, $J = 10.4$ Hz, H-3'), 5.53-5.44 (m, 3 H, H-5_{sp}, H-2' and H-4'), 5.29 (dd, 1 H, $J = 15.2, 7.6$ Hz, H-4_{sp}), 4.86 (d, 1 H, $J = 8.8$ Hz, NH_{sp}), 4.76 (d, 1 H, $J = 8.0$ Hz, H-1'), 4.47 (d, 1 H, $J = 11.2$ Hz, CH_{2-PMB-a}), 4.32-4.25 (m, 3 H, CH_{2-PMB-b}, H-1_{sp-a}, CH_{2-Fmoc-a}), 4.10-4.05 (m, 2 H, CH_{2-Fmoc-b} and CH_{Fmoc}), 3.94 (m, 1 H, H-5'), 3.85-3.70 (m, 6 H, OMe_{PMB}, H-1_{sp-b}, H-2_{sp} and H-3_{sp}), 3.53 (dd, $J = 13.6, 8.8$ Hz, H-6_{a'}), 3.38 (d, 1 H, $J = 12.0$ Hz, H-6_{b'}), 1.90 (m, 2 H, H-6_{sp}), 1.33-1.19 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.88 (t, 3 H, $J = 6.8$ Hz, H-18_{sp}); ^{13}C NMR (101 MHz, CDCl₃) δ 165.82, 165.43, 165.27 (3x C=O_{Bz}), 159.19 (C=O_{Fmoc}), 144.16, 144.00, 141.42, 141.37 (4x C_{q-Fmoc}), 137.39 (C-5_{sp}), 133.81, 133.44 (CH_{arom}), 130.54 (C_{q-arom}), 129.98, 129.86, 129.69, 129.51, 129.04, 128.85, 128.66, 128.55, 128.45, 127.80, 127.15 (CH_{arom} and C_{q-arom}), 125.30 (C-4_{sp}), 120.11, 120.08 (2x CH_{Fmoc}), 113.90, 113.83 (2x CH_{PMB}), 101.39 (C-1'), 79.10 (C-3_{sp}), 74.12 (C-5'), 72.59 (C-3'), 72.14 (C-2'), 70.14 (C-4'), 70.15 (CH_{2-PMB}), 68.44 (C-1_{sp}), 66.61 (CH_{2-Fmoc}), 55.37 (OMe_{PMB}), 53.83 (C-2_{sp}), 51.34 (C-6'), 47.33 (CH_{Fmoc}), 32.34 (C-6_{sp}), 29.83, 29.81, 29.78, 29.57, 29.49, 29.33, 29.26, 27.83, 22.82 (11x CH₂ C-7_{sp} to C-17_{sp}), 14.27 (C-18_{sp}); IR (neat): 2925, 2856, 2104, 1721, 1690, 1250, 1231, 1089, 1066, 1025 cm⁻¹; HRMS calculated for [C₆₈H₇₆N₄O₁₂ + H]⁺: 1141.5468, found 1141.5464.

6-deoxy-6-Azido-Glucosylsphingosine (28). General procedure for deprotection of protected glycosylsphingosine.



Yield (25 mg, 0.06 mmol, 77%). ^1H NMR (400 MHz, MeOD- d_4) δ 5.84 (dt, 1 H, $J = 15.2, 7.6$ Hz, H-5_{sp}), 5.48 (dd, 1 H, $J = 15.2, 6.8$ Hz, H-4_{sp}), 4.36 (d, 1 H, $J = 8.0$ Hz, H-1'), 4.28 (t, 1 H, $J = 5.6$ Hz, H-3_{sp}), 3.99 (m, 1 H, H-1_{a-sp}), 3.82 (d, 1 H, $J = 10.8$ Hz, H-1_{b-sp}), 3.54-3.24 (m, 7 H, H-2_{sp}, H-2', H-3', H-4' and H-5', H-6'), 3.08 (m, 2 H, H-6_{sp}), 1.41 (m, 2 H, H-7_{sp}), 1.36-1.28 (m, 20 H, H-8_{sp} to H-17_{sp}), 0.90 (t, 3 H, $J = 6.4$ Hz, H-18_{sp}); ^{13}C NMR (101 MHz, MeOD- d_4) δ 136.56 (C-5_{sp}), 128.57 (C-4_{sp}), 103.86 (C-1'), 77.54, 77.13, 74.83, 72.25 (C-2', C-3', C-4', C-5'), 71.36 (C-3_{sp}), 67.37 (C-1_{sp}), 56.27 (C-2_{sp}), 52.65 (C-6'), 33.07 (C-6_{sp}),

30.79, 30.76, 30.63, 30.47, 30.39, 30.16, 23.73 (11x CH₂ C-7_{sp} to C-17_{sp}), 14.45 (C-18_{sp}); IR (neat): 3300, 2917, 2850, 2100, 1668, 1434, 1201, 800, 721 cm⁻¹; HRMS calculated for [C₂₄H₄₆N₄O₆ + H]⁺: 487.3497, found 487.3506.

Galactosylsphingosine (30). See general procedure for the glycosylation of protected sphingosine **7** with

trichloro-imidate donor **29**. Yield (91 mg 0.075 mmol, 75%). R_f = 0.4 (20% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 8.09 (d, 2 H, J = 7.6 Hz, H_{arom}-OBz), 8.02 (d, 2 H, J = 7.2 Hz, H_{arom}-OBz), 7.99 (d, 2 H, J = 8.0 Hz, H_{arom}-OBz), 7.81-7.75 (m, 4 H, H_{arom}), 7.61 (t, 1 H, J = 7.6 Hz, H_{arom}), 7.55-7.18 (m, 19 H, H_{arom}), 6.83 (d, 2 H, J = 8.8 Hz, H_{PMB}), 6.01 (m, 1 H, H-4'), 5.77 (dd, 1 H, J = 10.4, 10.0 Hz, H-2'), 5.62 (dd, 1 H, J = 10.4, 3.2 Hz, H-3'), 5.44 (dt, 1 H, J = 15.6, 6.4 Hz, H-5_{sp}), 5.27 (dd, 1 H, J = 16.4, 8.0 Hz, H-4_{sp}), 4.90 (d, 1 H, J = 8.8 Hz, NH_{sp}), 4.75 (d, 1 H, J = 7.6 Hz, H-1'), 4.65 (dd, 1 H, J = 11.2, 6.4 Hz, CH₂-PMB), 4.48-4.43 (m, 2 H, CH₂-PMB, CH₂-Fmoc-a), 4.37-4.27 (m, 4 H, CH₂-Fmoc-b, H-1_{sp-ar}, H-5' and H-6_a), 4.12-4.05 (m, 2 H, H-6_b' and CH_{Fmoc}), 3.83-3.70 (m, 6 H, OMe_{PMB}, H-1_{sp-br}, H-2_{sp}, H-3_{sp}), 1.90 (m, 2 H, H-6_{sp}), 1.37-1.20 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.87 (t, 3 H, J = 7.2 Hz, H-18_{sp}); ¹³C NMR (101 MHz, CDCl₃) δ 166.15, 2x 165.64, 165.47 (4x C=O_{Bz}), 159.21 (C=O_{Fmoc}), 144.13, 143.95, 141.42, 141.37 (C_q-Fmoc), 137.32 (C-5_{sp}), 133.71, 133.43 (CH_{arom}), 130.50 (C_q-arom), 130.12, 129.89, 129.86, 129.42, 129.14, 128.88, 128.77, 128.61, 128.58, 128.42, 127.79, 127.15 (CH_{arom} and C_q-arom), 125.16 (C-4_{sp}), 120.08 (CH_{Fmoc}), 113.85 (CH_{PMB}), 102.04 (C-1'), 79.09 (C-3_{sp}), 71.63 (C-3'), 71.48 (C-5'), 70.28 (CH₂-PMB), 70.20 (C-2_{sp}), 68.61 (C-1_{sp}), 68.20 (C-4'), 66.54 (C-6'), 62.14 (CH₂-Fmoc), 55.31 (OMe_{PMB}), 53.97 (C-2_{sp}), 47.36 (CH_{Fmoc}), 32.35 (C-6_{sp}), 29.82, 29.78, 29.58, 29.33, 29.28, 22.82 (11x CH₂ C-7_{sp} to C-17_{sp}), 14.26 (C-18_{sp}). IR (neat): 2924, 2854, 1722, 1689, 1249, 1233, 1091, 1066, 1026 cm⁻¹; HRMS calculated for [C₇₅H₈₁NO₁₄ + H]⁺: 1220.5665, found 1220.5671.

Galactosylsphingosine (31). General procedure for deprotection of protected glycosylsphingosine. Yield (25 mg,

0.55 mmol, 74%). ¹H NMR (400 MHz, MeOD-*d*₄) δ 5.97 (dd, 1 H, J = 15.6, 6.6 Hz, H-5), 5.69 (dd, 1 H, J = 15.4, 6.9 Hz, H-4), 4.32 (d, 1 H, J = 7.6 Hz, H-1'), 4.09 (m, 1 H, H-3), 3.96 (t, 1 H, J = 6.4 Hz, H-5) 3.89-3.86 (m, 2 H, H-6'), 3.84-3.81 (m, 1 H, H-1_a), 3.77-3.70 (m, 2 H, H-1_b and H-2), 3.58- 3.48 (m, 3 H, H-2', H-3' and H-4'), 2.05 (m, 2 H, H-6), 1.48 (m, 2 H, H-7), 1.33-1.23 (m, 20 H, H-8 to H-17), 0.90 (t, 3 H, J = 6.8 Hz, H-18); ¹³C NMR (101 MHz, MeOD-*d*₄) δ 141.73 (C-5), 123.06 (C-4), 104.42 (C-1'), 77.00 (C-4'), 74.72 (C-3'), 72.46 (C-2'), 70.67 (C-6'), 70.34 (C-3), 62.67 (C-1), 54.07 (C-2), 33.07 (C-6), 30.79, 30.76, 30.47, 26.52, 23.73 (11x CH₂ C-7 to C-17), 14.44 (C-18); IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm⁻¹; HRMS calculated for [C₂₄H₄₇NO₇ + H]⁺: 462.3431, found 461.3425.

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Chapter 4

Synthesis of a Panel of Carbon-13-Labeled Phosphosphingolipids

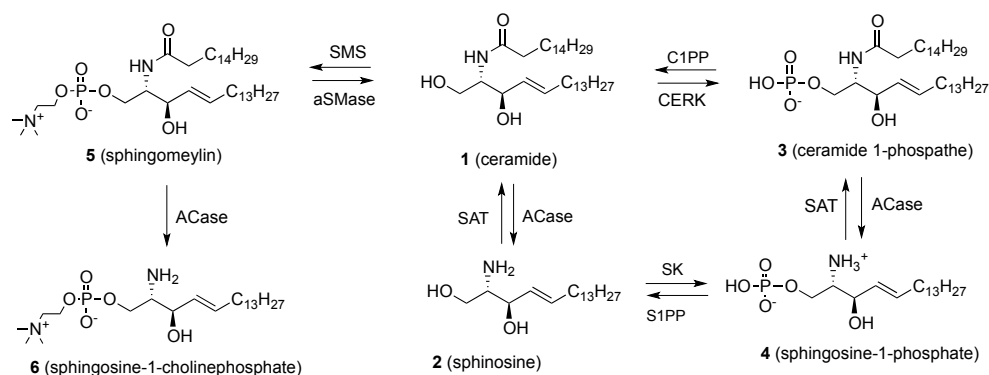
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4.1 Introduction

Chapter 2 described the synthesis of carbon-13-labeled glycosphingolipids.^[1] Another class of functionalized sphingolipids are the phospholipids, metabolites often encountered together with glycosphingolipids. As well, the metabolism of both sphingolipid families is often interconnected.^[2,3] Some major phosphosphingolipids encountered in mammalian cells, and their biosynthetic pathways starting from ceramide (**1**) are shown in Figure 4.1.

Ceramide kinase (CERK) catalyzes phosphorylation of ceramide (**1**), using ATP as phosphate source, to form ceramide-1-phosphate (C1P, **3**). The reverse reaction, dephosphorylation of C1P (**3**) to give ceramide is catalyzed by ceramide-1-phosphate phosphatase (C1PP). Alternatively, acid ceramidase (ACase) may take on C1P (**3**) as substrate to produce, by hydrolysis of the amide bond and concomitant release of the fatty acid, sphingosine-1-phosphate **4** (S1P). S1P, an important signaling lipid, is normally produced from sphingosine (**2**) by sphingosine kinase (SK) mediated phosphorylation of the primary alcohol. Sphingosine-1-phosphate phosphatase (S1PP) in turn produces sphingosine **2** from S1P (**4**).



Scheme 4.1 Partial overview of phosphosphingolipid metabolism in man. ACase: acid ceramidase; aSMase: acid sphingomyelinase; C1PP: ceramide-1-phosphate phosphatase; CERK: ceramide kinase; S1PP: sphingosine-1-phosphate phosphatase; SAT: sphingosine acyl transferase; SMS: sphingomyelin synthase.

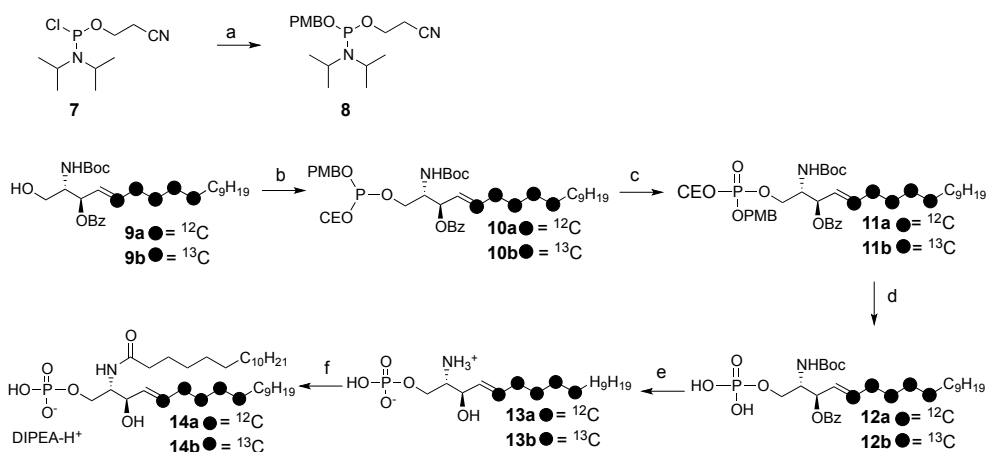
Another occurring phosphosphingolipid is sphingomyelin (**5**), a zwitterionic species composed of ceramide, the primary alcohol of which carries a choline phosphate group. Sphingomyelin synthase (SMS) converts ceramide to sphingomyelin using phosphatidylcholine as the choline phosphate donor in a transesterification process. Acid sphingomyelinase (aSMase) hydrolyzes sphingomyelin to ceramide and phosphocholine. Inherited, genetic deficiency in aSMase leads to accumulation of sphingomyelin in the lysosomes. This deficiency is caused by mutation of SMPD1 gene, leading to the lysosomal storage disorders, Niemann-Pick disease A and B.^[5] Carbon-13-labeled sphingomyelin would be an excellent tool to determine sphingomyelin levels^[6] and to identify potential alternative metabolic pathways in these disorders.^[7] It has recently become clear that the primary storage material in Niemann-Pick patients, sphingomyelin (**5**) may be processed by ACase to produce sphingosine-1-cholinephosphate (**6**) as a secondary storage material.^[8] This situation resembles earlier findings in relation to Gaucher disease (processing of glucosylceramide – the primary storage material – into glucosylsphingosine) and Fabry disease (globotriaosylceramide as the primary storage material is partially processed to globotriaosylsphingosine). It has been argued that in Gaucher and Fabry

alike, these secondary storage lysolipids may contribute to development of the diseases.^[9] As well, very recently it has become clear that glucosylsphingosine, likely produced through the action of ceramidase (ACase), hardly present in healthy individuals and markedly increased in Gaucher patients, can give rise to plasma cells producing antibodies elicited against these lysolipids. Plasma cells that in turn may transform into malignant cells that eventually lead to multiple myeloma.^[10] A panel of carbon-13-labeled phosphosphingolipids would be highly useful tools to study biosynthesis and degradation pathways of these molecules in health and disease. The synthesis of such a stable isotope metabolite panel is described in this Chapter and builds on the research described in Chapter 2, specifically the synthesis of ¹³C-labeled, protected sphingosine as a common building block.

4.2 Results and discussion

Partially protected sphingosine **9a/b** (Chapter 2),^[1] with the primary alcohol free for modification, bearing protective groups compatible with phosphoramidite chemistry,^[12–14] and incorporating either zero carbon-13 isotopes (**9a**) or five carbon-13 isotopes (**9b**) served as the starting point of the synthetic efforts. The required phosphorylating agent, phosphoramidite (**8**), was synthesized from 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidate **7** using 4-methoxy benzyl alcohol and

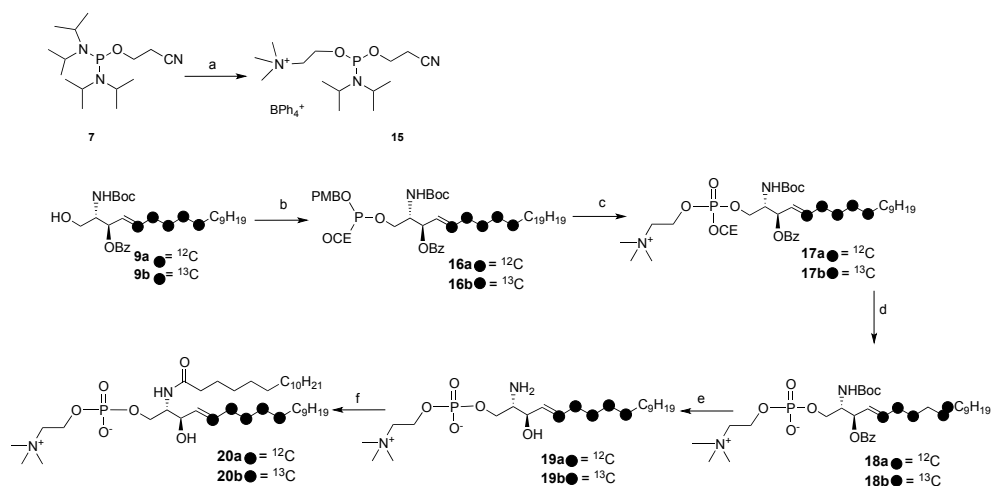
Scheme 4.1 Synthesis of (¹³C₅-labeled) sphingosine 1-phosphate **13a/b** and ceramide 1-phosphate **14a/b**.



Reagents and conditions: (a) PMBOH, DIPEA, DCM, r.t., 1 h, 82%; (b) **8**, tetrazole, MeCN, r.t., 1 h; (c) tBuOOH, r.t., 30 min; (d) (i) DBU, r.t., 1 h; (ii) AcOH:H₂O (2:1), r.t., 2 days; (e) NaOMe, MeOH, r.t., 3 days; (vi) TFA:DCM (1:1), r.t., 1 h, **13a**: 68%, **13b**: 76% (over four steps); (f) (i) BSA, r.t., 20 h; (ii) palmitoyl chloride, DIPEA, DCM, 0 °C to r.t., 2 h, **14a**: 79%, **14b**: 81%.

DIPEA in dry DCM in 82% yield. Phosphoramidite **8** and protected sphingosine **9a/b** were coupled using tetrazole as the activator, giving the corresponding phosphite triester **10a/b**, which was subsequently oxidized (treatment with *t*BuOOH) without intermediate work-up and purification to give fully protected sphingosine 1-phosphate **11a/b**. Treatment with DBU led to removal of the 2-cyanoethyl group in the phosphotriester, after which treatment with mild acid (acetic acid in water) gave phosphate **12a/b**. Finally, treatment with sodium methoxide in methanol, followed by treatment with TFA led to methanolysis of the benzoyl ester and removal of the N-Boc group, respectively, to yield the target phosphosphingolipids **13a/b**. Purification of crude sphingosine-1-phosphate **13a/b** proved complicated due to insolubility in solvent systems normally used in either silica gel chromatography or reverse-phase HPLC. Dissolving crude **13a/b** in boiling acetic acid followed by addition of water led to precipitation of the product.^[12,14] The precipitate was filtered successively and washed with water (to remove salts), followed by organic solvents (MeOH, DCM, acetone and diethyl ether to remove remaining organic impurities), giving analytically pure sphingosine-1-phosphate **13a/b** in 68% (**13a**) and 76% (**13b**) yield, respectively (for the same reason – insolubility in most organic solvents – NMR spectra of **13a/b** were recorded in deuterated acetic acid^[14]). Besides the utility as internal standards for metabolomics applications, sphingosine-1-phosphates **13a/b** also served as starting point to produce the corresponding ceramide-1-phosphates **14a/b**. In the first instance, subjecting **13a/b** to Schotten-Bauman conditions (reaction of the free amine in **13a/b** with palmitoyl chloride) appeared an unsuitable strategy, again because the starting material does not dissolve in suitable solvent systems. Treatment of **13a/b** with *N,O*-bis(trimethylsilyl)acetamide (BSA – a strong trimethylsilylation reagent) followed by treatment with palmitoyl chloride and aqueous work-up (at which stage the intermediate trimethylsilyl protective groups are removed) gave ceramide-1-phosphates **14a/b** as the DIPEA salt in good yields.^[16]

The synthesis of sphingosine-1-cholinephosphates **19a/b** and sphingomyelins **20a/b** followed strategies (see Scheme 4.2) essentially the same as for compounds **13a/b** and **14a/b**, but now starting from phosphoramidite **15**.^[17,18] It was decided to introduce the choline moiety already in the phosphoramidite stage and not following the phosphorylation event, to avoid unnecessarily harsh conditions in later stages of the synthesis. Known literature procedures on the synthesis of phosphocholine moieties are based on either tosylate^[17] or hexafluorophosphate^[18] as counter-ions for the quaternary ammonium ion. However, ammonium salts composed of these cations are often poorly soluble in DCM. For this reason, tetraphenylborate was selected as the counter-ion. For the coupling with sphingosine **9a/b** and phosphoramidite **15**, tetrazole activation (see Scheme 4.2) conditions were used, but the solvent was switched from DCM to MeCN. After confirming the formation of phosphite trimer **16a/b** (³¹P NMR: 140 ppm), *t*BuOOH was added to oxidize the phosphite triester **16a/b** forming **17a/b**.

Scheme 4.2 Synthesis of ($^{13}\text{C}_5$ -labeled) sphingosine 1-cholinephosphate **19a/b** and sphingomyelins **20a/b**.


Reagents and conditions: (a) choline tetraphenylborane, diisopropylammonium tetrazolide DCM:MeCN (2:1), r.t., 2 h, quant; (b) **15**, tetrazole, MeCN, r.t., 1 h; (c) tBuOOH, r.t., 30 min.; (d) DBU, r.t., 1 h; (e) (i) NaOMe, MeOH, r.t., 3 days; (ii) TFA:DCM (1:1), r.t., 1 h, **19a**: 59%, **19b**: 56% (four steps); (f) (i) BSA, r.t., 20 h; (ii) palmitoyl chloride, DIPEA, DCM, 0 °C to r.t., 2 h, **20a**: 61%, **20b**: 66%.

The fully protected sphingosine-1-cholinephosphate **17a/b** was deprotected, by first removing the protecting group on the choline phosphate (2-cyanoethyl) (DBU) giving the zwitter-ionic sphingosine **18a/b**. Next, the sphingosine part was deprotected. To this end the benzoyl was removed using NaOMe in methanol, followed by acid treatment (TFA) to deprotect the amine giving crude sphingosine 1-choline phosphate **19a/b**. Due to the better solubility compared to sphingosine 1-phosphate **13a/b**, the crude mixture was purified by HPLC-MS giving the pure zwitter-ionic sphingosines **19a/b**. Schotten-Bauman conditions were tried to get the corresponding ceramides **20a/b**. Unfortunately, these conditions were not suitable to acylate the amine. Therefore the same conditions as described for the acylation of sphingosine-1-phosphate were applied giving after silica gel chromatography the corresponding sphingomyelin **20a/b** in a yield of 61-66%.^[13]

4.3 Conclusion

The synthesis of a comprehensive set of carbon-13-labeled phosphosphingolipids, together with their non-isotopically-enriched form, is described in this Chapter. The synthetic strategy is based on phosphoramidite chemistry to install either a phosphate (as in **11a/b**) or a choline phosphate (**13a/b**). The strategy proved successful, but measures needed to be taken to deal with solubility issues in both syntheses. With these carbon-13-labeled phosphosphingolipids in hand, improved mass spectrometric procedures can be developed for phosphosphingolipids to determine the levels in biological materials and

giving better understanding of the metabolism of phosphosphingolipids.^[6] For the synthetic procedure that was developed, new phosphorylating agents were introduced. With the new phosphorylation reagents **8** and **15** it is possible to introduce (choline)phosphate diesters under mild conditions and in high yield.

4.4 Experimental Section

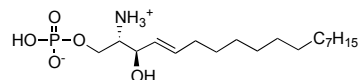
General Remarks: [¹³C₂]-acetic acid (99.95% isotopically pure, product code CLM-105), potassium [¹³C]-cyanide (99% isotopically pure, product code CLM-297), and [1,2,3-¹³C₃]-myristic acid (99% isotopically pure, product code CLM-3665) were purchased from Cambridge Isotope Laboratories, Inc., and were used as received. Commercially available reagents and solvents (Acros, Fluka, or Merck) were used as received, stated otherwise stated. CH₂Cl₂ and THF were freshly distilled before use, over P₂O₅ and Na/benzophenone, respectively. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide. Traces of water were removed from starting compounds by coevaporation with toluene. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out using airflow of the indicated solvent systems on Screening Devices Silica gel 60 (40–63 µm mesh). Size-exclusion chromatography was carried out on Sephadex LH20 (MeOH/CH₂Cl₂, 1:1). Analytical TLC was carried out on aluminum sheets (Merck, silica gel 60, F254). Compounds were visualized by UV absorption (254 nm), or by spraying with ammonium molybdate/cerium sulphate solution [(NH₄)₆Mo₇O₂₄·4H₂O (25 g/L), (NH₄)₄Ce(SO₄)₆·2H₂O (10 g/L), 10 % sulphuric acid in ethanol] or phosphormolybdic acid in EtOH (150 g/L), followed by charring (ca. 150 °C). IR spectra were recorded with a Shimadzu FTIR-8300 instrument and are reported in cm⁻¹. Optical rotations were measured with a Propol automatic polarimeter (sodium D-line, λ = 589 nm). ¹H and ¹³C NMR spectra were recorded with a Bruker AV 400 MHz spectrometer at 400.2 (¹H) and 100.6 (¹³C) MHz, or with a Bruker AV 600 MHz spectrometer at 600.0 (¹H) and 151.1 (¹³C) MHz. Chemical shifts are reported as δ values (ppm), and were referenced to tetramethylsilane (δ = 0.00 ppm) directly in CDCl₃, or using the residual solvent peak (D₂O). Coupling constants (*J*) are given in Hz, and all ¹³C spectra were proton decoupled. NMR assignments were made using COSY and HSQC, and in some cases TOCSY experiments. LC–MS analysis was carried out with an LCQ Advantage Max (Thermo Finnigan) instrument equipped with a Gemini C18 column (Phenomenex, 50, 4.6 mm, 3 µm), using the following buffers: A: H₂O, B: acetonitrile, and C: aq. TFA (1.0 %). HPLC–MS purifications were carried out with an Agilent Technologies 1200 Series automated HPLC system with a Quadrupole MS 6130, equipped with a semi-preparative Gemini C18 column (Phenomenex, 250×10.00, 5µm). Products were eluted using the following buffers: A: aq. TFA (0.2 %), B: acetonitrile (HPLC-grade), 5 mL/min. Purified products were lyophilized with a CHRIST ALPHA 2–4 LDPLUS apparatus to remove water and traces of buffer salts.

4-methoxybenzyl-2-cyanoethyl *N,N*-diisopropylphosphoramidate (**8**). 2-Cyanoethyl *N,N*-

diisopropylchlorophosphoramidate **7** (0.89 mL, 4.0 mmol, 1.0 eq) was dissolved in dry DCM (12 mL) under protected atmosphere and DIPEA (1.04 mL, 6.0 mmol, 1.5 eq) was added, followed by the addition of 4-methoxybenzyl alcohol (0.5 mL, 4.0 mmol, 1 eq). The reaction was stirred at room temperature for 1 hour. The reaction mixture was then transferred to an extraction funnel with EtOAc (100 mL) and washed twice with sat. aq. NaHCO₃ (100 mL) and brine (100 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics dried (Na₂SO₄), filtered and concentrated *in vacuo*. Purification by column chromatography (10% DCM, 1% NEt₃, in pentane to 10% DCM, 10% EtOAc, 1% NEt₃, in pentane) giving titled compound **8** as a clear oil (1.11 g, 3.28 mmol, 82%). *R*_f = 0.2 (10% DCM, 10% EtOAc, 1% NEt₃, in pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.27 (d, 2 H, *J* = 4.4 Hz, 2x H_{PMB}), 6.87 (d, 2 H, *J* = 4.4 Hz, 2x H_{PMB}), 4.69 (dd, 1 H, *J* = 9.0, 8.4 Hz, CH₂-PMB-a), 4.60 (dd, 1 H, *J* = 9.2, 9.0 Hz, CH₂-PMB-b), 3.83 (m, 2 H, -OCH₂-), 3.81 (s, 3 H, OMe_{PMB}), 3.60 (m, 2 H, 2x CH₂-diisopropyl), 2.61 (t, 2 H, *J* = 6.4 Hz, -CH₂-CN), 1.19 (t, 12 H, *J* = 7.2 Hz, 4x CH₂-diisopropyl); ¹³C NMR (101 MHz, CDCl₃) δ 159.1 (C_q-PMB), 131.2 (C_q-PMB), 128.7 (CH_{arom}-

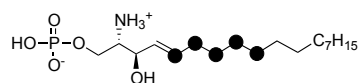
PMB), 117.7 (CN), 113.8 (CH_{arom}-PMB), 65.2 (d, *J* = 1.8 Hz, CH₂-PMB), 58.4 (d, *J* = 1.9 Hz, CH₂-OCE), 55.3 (OMe-PMN), 43.2, 43.1 (2x CH_{diisopropyl}), 24.72, 24.65, 24.62, 24.55 (4x CH₃-Diisopropyl), 20.3 (CH₂CN); ³¹P NMR (162 MHz, CDCl₃) δ 148.5.

1-Phosphate-Sphingosine (13a). Sphingosine acceptor (**9a**) (50 mg, 0.1 mmol, 1.0 eq) was co-evaporated twice in



toluene (2.5 mL) and then dissolved in anhydrous MeCN (4 mL) under protected atmosphere. 2-Cyanoethoxy-diisopropylamino-4-methoxy-benzyloxy-phosphine **8** (40 mg, 0.12 mmol, 1.2 eq) was added followed by the addition of 4,5-dicyanoimidazole (23.6 mg, 0.2 mmol, 2.0 eq). The reaction was stirred until TLC showed complete conversion of the sphingosine acceptor **9a** (~1 hour) and with ³¹P NMR (140 ppm). Anhydrous t-BuOOH (~5.5 M in nonane) (91 µL, 0.5 mmol, 5.0 eq) was added and the reaction was stirred (~30 minutes) at room temperature until ³¹P NMR (-1.7 ppm) showed full conversion. DBU (76 µL, 0.5 mmol, 5.0 eq) was added and stirred (~1 hour) at room temperature until HPLC-MS showed full removal of 2-Cyanoethoxy group. The reaction was concentrated *in vacuo* and coevaporated twice with toluene (20 mL). The crude reaction mixture was then dissolved in AcOH:H₂O (95:5) (5 mL) and stirred (~2 days) at room temperature until LC-MS showed full removing of 4-methoxy-benzyloxy group. The crude reaction mixture was concentrated *in vacuo* and coevaporated twice with toluene (10 mL). The residue was dissolved in methanol (2.5 mL) and sodium methoxide (30% in methanol) (0.26 mL, 2.0 mmol, 20 eq) was added. The reaction was stirred for 3 days at room temperature and the progression of the reaction was followed by HPLC-MS. The reaction was neutralized with Amberlite H⁺ resin and the reaction mixture was filtered and concentrated *in vacuo*. The crude reaction mixture was coevaporated in toluene (10 mL) and put on ice-bath before addition of DCM (1 mL) and TFA (1 mL). The reaction mixture was stirred for 5 minutes at 0°C. The solution was diluted with toluene (10 mL) and concentrated to about 2 mL *in vacuo*. The coevaporation was repeated two times with toluene (10 mL), before concentration to dryness. The residue was dissolved in hot glacial acetic acid followed by addition of water. The precipitate was filtered and washed with water, acetone and diethylether giving pure sphingosine-1-phosphate **13a** as white powder (29 mg, 0.07 mmol, 76%). ¹H NMR (600 MHz, Acetic acid-*d*₃) δ 5.92 (dt, 1 H, *J* = 14.3, 6.7 Hz, H-5), 5.55 (dd, 1 H, *J* = 15.5, 7.0 Hz, H-4), 4.50 (t, 1 H, *J* = 6.1 Hz, H-3), 4.25 (m, 2 H, H-1), 3.70 (m, 1 H, H-2), 2.09 (q, 2 H, *J* = 7.5 Hz, H-6), 1.41 (m, 2 H, H-7), 1.36-1.23 (m, 20 H, H-8 to H-17), 0.89 (t, 3 H, *J* = 7.2 Hz, H-18); ¹³C NMR (151 MHz, Acetic acid-*d*₃) δ 137.6 (C-5), 126.8 (C-4), 70.7 (C-3), 62.8 (C-1), 57.2 (C-2), 33.6 (C-6), 32.7, 30.58, 30.56 x3, 30.52, 30.51, 30.42, 30.23, 30.16, 29.68, 23.47 (11x CH₂ C-7 to C-17), 14.4 (C-18); ³¹P NMR (162 MHz, Acetic acid-*d*₃) δ -0.51; IR (neat): 3427, 2950, 2847, 1634, 1543, 1460, 1246, 1066, 1029, 925 cm⁻¹; HRMS calculated for [C₁₈H₃₈NO₅P + H]⁺: 380.2568, found 380.2575.

1-Phosphate-[5,6,7,8,9-¹³C₅]-sphingosine (13b). Sphingosine acceptor (**9b**) (35 mg, 69 µmol, 1.0 eq) was co-



evaporated twice in toluene (2.5 mL) and then dissolved in anhydrous MeCN (3 mL) under protected atmosphere. 2-Cyanoethoxy-diisopropylamino-4-methoxy-benzyloxy-phosphine **8** (28 mg, 83 µmol, 1.2 eq) was added followed by the addition of 4,5-dicyanoimidazole (16.2 mg, 0.14 mmol, 2.0 eq). The reaction was stirred until TLC showed complete conversion of the sphingosine acceptor (~1 hours) and with ³¹P NMR (140 ppm). Anhydrous t-BuOOH (~5.5 M in nonane) (63 µL, 0.35 mmol, 5.0 eq) was added and the reaction was stirred (~30 minutes) at room temperature until ³¹P NMR (-1.7 ppm) showed full conversion. DBU (52 µL, 0.34 mmol, 5.0 eq) was added and stirred (~1 hour) at room temperature until HPLC-MS showed full removing of 2-Cyanoethoxy group. The reaction was concentrated *in vacuo* and coevaporated twice with toluene (20 mL). The crude reaction mixture was then dissolved in AcOH:H₂O (95:5) (4 mL) and stirred (~2 days) at room temperature until HPLC-MS showed full removing of 4-methoxy-benzyloxy group. The crude reaction mixture was concentrated *in vacuo* and coevaporated twice with toluene (10 mL). The residue was dissolved in methanol (2.0 mL) and sodium methoxide (30% in methanol) (0.18 mL, 1.4 mmol, 20 eq) was added. The reaction was stirred for 3 days at room temperature and the progression of the reaction was followed by HPLC-MS. The reaction was neutralized with Amberlist H⁺ resin and the reaction mixture was filtered and concentrated *in vacuo*. The crude reaction mixture was coevaporated in toluene (10 mL) and put on ice-bath before addition of DCM (1 mL) and TFA (1 mL). The reaction mixture was stirred for 5 minutes at 0°C. The

solution was diluted with toluene (10 mL) and concentrated to about 2 mL *in vacuo*. The coevaporation was repeated two times with toluene (10 mL), before concentration to dryness. The residue was dissolved in hot glacial acetic acid followed by addition of water. The precipitate was filtered and washed with water, acetone and diethylether giving pure sphingosine-1-phosphate as white powder (20 mg, 0.05 mmol, 76%). ^1H NMR (600 MHz, Acetic acid- d_3) δ 5.91 (dm, 1 H, J = 152.9 Hz, H-5, ^{13}C), 5.55 (m, 1 H, H-4), 4.49 (q, 1 H, J = 5.4 Hz, H-3), 4.25 (m, 2 H, H-1), 3.70 (m, 1 H, H-2), 2.09 (dm, 2 H, J = 126.9 Hz, H-6, ^{13}C), 1.56-1.13 (m, 22 H, H-7 to H-17, $3\times^{13}\text{C}$), 0.89 (t, 3 H, J = 7.2 Hz, H-18); ^{13}C NMR (150 MHz, Acetic acid- d_3) δ 137.7 (d, J = 45.0 Hz, C-5, ^{13}C), 128.5 (dd, J = 72.4, 3.5 Hz C-4), 70.8 (d, J = 5.1 Hz, C-3), 62.8 (C-2), 57.2 (C-1), 33.1 (dd, J = 42.4, 32.3 Hz, C-6, ^{13}C), 30.8-29.2 (m, $10\times\text{CH}_2$, $3\times^{13}\text{C}$), 23.5 (CH_2), 14.3 (C-18); ^{31}P NMR (Acetic acid- d_3) δ -0.51; IR (neat): 3428, 2949, 2847, 1634, 1542, 1460, 1246, 1066, 1029, 925 cm^{-1} . HRMS calculated for $[\text{C}_{13}^{13}\text{C}_5\text{H}_{38}\text{NO}_5\text{P} + \text{H}]^+$: 385.2213, found 385.2215.

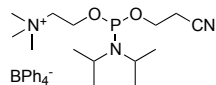
Ceramide-1-phosphate (DIPEA salt) (14a). Sphingosine **13a** (9 mg, 0.025 mmol, 1 eq) was mixed with BSA (0.3 mL) under protected atmosphere and stirred overnight at room temperature. The mixture was concentrated in high vacuum and the silylated phosphosphingosine was dissolved in dry DCM (0.3 mL) under a protected atmosphere and cooled to 0 $^{\circ}\text{C}$. To the solution was added DIPEA (13 μL , 0.075 mmol, 3.0 eq) followed by palmitoyl chloride (9 μL , 0.03 mmol, 1.2 eq). The reaction was left stirring,

reaching room temperature over 2 h. The reaction mixture was then concentrated *in vacuo* and the residue was dissolved in a small amount of MeOH/DCM. Precipitated with acetone gave ceramide-1-phosphate **14a** (12 mg, 0.02 mmol, 79%). ^1H NMR (600 MHz, MeOD- d_4) δ 5.70 (dt, 1 H, J = 15.6, 8.4 Hz, H-5), 5.45 (dd, 1 H, J = 15.0, 8.8 Hz, H-4), 4.18 (m, 1 H, H-1_a), 4.10 (t, 1 H, J = 7.2 Hz, H-3), 3.95-3.92 (m 2 H, H-1_b and H-2), 3.68 (m, 1 H, DIPEA), 3.17 (m, 2 H, DIPEA), 2.18 (m, 2 H, H-2*), 2.02 (m, 2 H, H-6), 1.58 (m, 2 H, H-7), 1.38-1.28 (m, 48 H, H-8 to H-17, H-3* to H-15* and DIPEA), 0.89 (t, 6 H, J = 7.2 Hz, H-18 and H-16*); ^{13}C NMR (150 MHz, MeOD- d_4) δ 175.9 (C=O), 134.9 (C-5), 130.9 (C-4), 72.3 (C-3), 65.4 (C-1), 55.7 (C-2), 55.2 (CH, DIPEA), 37.3 (C-2*), 43.67 (CH_2 DIPEA), 33.4, 33.0, 30.76, 30.72 $\times 7$, 30.71, 30.69 $\times 3$, 30.68, 30.67, 30.65, 30.59, 30.52, 30.39, 30.37, 30.35, 30.35, 30.31, 27.03, (25 $\times\text{CH}_2$ C-6 to C-17 and C-3* to C-15*), 18.7, 17.2 (CH_3 DIPEA), 14.4 (C-18 and C-16*); ^{31}P NMR (162 MHz, MeOD- d_4) δ -0.53; IR (neat): IR (neat): 3429, 2916, 2847, 1738, 1633, 1543, 1460, 1247, 1065, 1029, 925 cm^{-1} ; HRMS Calculated for $[\text{C}_{34}\text{H}_{68}\text{NO}_6\text{P} + \text{H}]^+$: 618.4864, found 618.4865.

1-Phosphate-2-N-(hexadecanoyl)-[5,6,7,8,9- $^{13}\text{C}_5$]-sphingosine (14b). Sphingosine **13b** (12 mg, 0.03 mmol, 1 eq) was mixed with BSA (0.4 mL) under protected atmosphere and stirred over night at room temperature. The mixture was concentrated under high vacuum and the silylated phosphosphingosine was dissolved in dry DCM (0.4 mL) under a protected atmosphere and cooled to 0 $^{\circ}\text{C}$. To the solution was added

DIPEA (16 μL , 0.1 mmol, 3.0 eq) followed by palmitoyl chloride (12 μL , 0.04 mmol, 1.2 eq). The reaction was left stirring, reaching room temperature over 2 h. The reaction mixture was then concentrated *in vacuo* and the residue was dissolved in a small amount of MeOH/DCM and precipitated with acetone gave 1-phosphate-ceramide **14b** (16 mg, 0.03 mmol, 81%); ^1H NMR (600 MHz, MeOD- d_4) δ 5.66 (dm, 1 H, J = 156 Hz, H-5), 5.44 (m, 1 H, H-4), 4.18 (m, 1 H, H-1_a), 4.09 (m, H-3), 3.94-3.91 (m, 2 H, H-1_b and H-2), 3.68 (m, 1 H, DIPEA), 3.17 (m, 2 H, DIPEA), 2.17 (t, 2 H, J = 7.2 Hz, H-2*), 2.01 (dm, 2 H, J = 126.0 Hz, H-6), 1.57-1.17 (m, 48 H, H-7 to H-17, H-3* to H-15* and DIPEA), 0.88 (t, 6 H, J = 6.6 Hz, H-18 and H-16*); ^{13}C NMR (150 MHz, MeOD- d_4) δ 175.6 (C=O), 135.9 (d, J = 42.0 Hz, C-5), 130.4 (d, J = 72.0 Hz, C-4), 72.1 (d, J = 4.5 Hz, C-3), 65.3 (d, J = 4.5 Hz, H-1), 55.4 (C-2), 37.1 (C-2*), 33.5-32.9 (m) 30.6-29.8 (m), 26.8 (25 $\times\text{CH}_2$ C-6 to C-17 and C-3* to C-15*), 18.7, 17.2 (CH_3 DIPEA), 14.4 (C-18 and C-16*); ^{31}P NMR (162 MHz, MeOD- d_4) δ -0.51; IR (neat): 3430, 2914, 2847, 1726, 1636, 1543, 1458, 1246, 1066, 1029, 925 cm^{-1} ; HRMS Calculated for $[\text{C}_{29}^{13}\text{C}_5\text{H}_{68}\text{NO}_6\text{P} + \text{H}]^+$: 619.4509, found 619.4512.

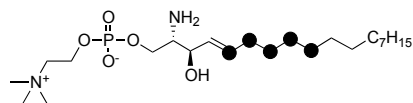
Choline-2-cyanoethyl *N,N*-diisopropylphosphoramidate tetraphenylborate (15). 2-cyanoethyl *N,N,N,N'*-tetraisopropylphosphordiamidite **7** (0.30 g, 1.0 mmol, 1.0 eq) was dissolved in DCM:MeCN (2:1, 3 mL) under protected atmosphere and diisopropyl ammonium tetrazolide (0.17 g, 0.5 mmol, 0.5 eq) was added. Choline tetraphenylborate (0.42 g, 1.0 mmol, 1.0 eq)^[19] was added and the reaction was stirred at room temperature for



2 hours. The reaction mixture was diluted with DCM (10 mL) and was washed twice with sat. aq. NaHCO_3 (10 mL). The aqueous layers were extracted with DCM (10 mL) and the combined organics dried (Na_2SO_4), filtered and concentrated *in vacuo* giving a brownish solid which is used without further purification. ^1H NMR (400 MHz, CDCl_3) δ 7.54-7.45 (m, 8 H, H_{arom}), 7.04 (t, 8 H, $J = 7.6$ Hz, H_{arom}), 6.88 (t, 4 H, $J = 7.2$ Hz, H_{arom}), 3.70 (m, 1 H, $-\text{OCH}_2\text{-a-OCE}$), 3.56 (m, 1 H, $-\text{OCH}_2\text{-b-OCE}$), 3.50 (m, 2 H, 2x $\text{CH}_{\text{diisopropyl}}$), 3.24 (m, 2 H, $-\text{OCH}_2\text{-choline}$), 2.44 (t, 2 H, $J = 6.0$ Hz, $-\text{CH}_2\text{CN}$), 1.71 (m, 2 H, $-\text{CH}_2\text{NMe}_3$), 1.66 (s, 9 H, 3x $\text{Me}_{\text{choline}}$), 1.17, 1.15, 1.14, 1.13 (4x s, 3 H, $\text{CH}_3\text{-diisopropyl}$); ^{13}C NMR (100 MHz, CDCl_3) δ 165.3, 164.8, 164.3, 163.8 (4x $\text{C}_{\text{q-arom}}$), 136.2 (CH_{arom}), 126.1 (CH_{arom}), 122.1 (CH_{arom}), 118.0 (CN), 65.5 ($-\text{CH}_2\text{CN}$), 58.0 ($-\text{OCH}_2\text{-OCE}$), 57.7 ($-\text{OCH}_2\text{-choline}$), 53.2, (3x $\text{CH}_3\text{-choline}$), 43.4, 43.4, (2x $\text{CH}_{\text{isopropyl}}$), 24.8, 2x 24.71, 24.64 (4x $\text{CH}_3\text{-isopropyl}$), 20.4 ($-\text{CH}_2\text{CN}$); ^{31}P (162 MHz, CDCl_3) δ 148.1.

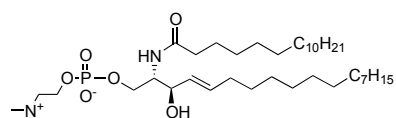
Sphingosine-1-cholinephosphate (19a) Sphingosine acceptor (**9a**) (0.213 g, 0.42 mmol, 1.0 eq) was co-evaporated twice in toluene (10 mL) and then dissolved in anhydrous MeCN (17 mL) under protected atmosphere. 2-Choline-2-cyanoethyl *N,N*-diisopropylphosphoramidate tetraphenylborate (**15**) (530 mg, 0.85 mmol, 2.0 eq) was added followed by the addition of tetrazole (0.45 M in MeCN) (0.93 mL, 0.42 mmol, 1.0 eq). The reaction was stirred until TLC showed complete conversion of the sphingosine acceptor (~2 hours). Anhydrous *t*-BuOOH (~5.5 M in nonane) (0.38 mL, 2.10 mmol, 5.0 eq) was added and the reaction was stirred (~30 minutes) at room temperature until ^{31}P NMR showed full conversion. DBU (0.31 mL, 2.10 mmol, 5.0 eq) was added and stirred (~1 hour) at room temperature until HPLC-MS showed full removal of 2-cyanoethoxy group. The reaction was concentrated *in vacuo* and coevaporated twice with toluene (20 mL). The crude reaction mixture was then dissolved in methanol (10 mL) and sodium methoxide (30% in methanol) (1.1 mL, 8.4 mmol, 20 eq) was added. The reaction was stirred for 3 days at room temperature and the progression of the reaction was followed by HPLC-MS. The reaction was neutralized with Amberlite H^+ resin and the reaction mixture was filtered and concentrated *in vacuo*. The crude reaction mixture was coevaporated in toluene (10 mL) and put on ice-bath before addition of DCM (5 mL) and TFA (5 mL). The reaction mixture was stirred for 5 minutes at 0°C . The solution was diluted with 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 reaction progress was monitored by HPLC-MS and purified with HPLC-MS as well yielding **19a** (0.11 g, 0.25 mmol, 59%). ^1H NMR (600 MHz, $\text{MeOD-}d_4$) δ 5.89 (dt, 1 H, $J = 15.2, 6.7$ Hz, H-5), 5.49 (dd, 1 H, $J = 15.2, 6.9$ Hz, H-4), 4.29 (m, 3 H, H-3 and $\text{CH}_2\text{-choline}$), 4.12 (m, 1 H, H-1_a), 4.03 (m, 1 H, H-1_b), 3.65 (m, 2 H, $\text{CH}_2\text{-choline}$), 3.37 (m, 1 H, H-2), 3.23 (s, 9 H, $\text{CH}_3\text{-choline}$), 2.11 (q, 2 H, $J = 7.5$ Hz, H-6), 1.43 (m, 2 H, H-7), 1.37-1.20 (m, 20 H, H-8 to H-17), 0.89 (t, 3 H, $J = 6.8$ Hz, H-18); ^{13}C NMR (151 MHz, $\text{MeOD-}d_4$) δ 137.2 (C-5), 128.2 (C-4), 70.7 (C-3), 67.4 (m, $\text{CH}_2\text{-choline}$), 63.6 (d, $J = 5.1$ Hz, C-1), 60.6 (d, $J = 5.0$ Hz, $\text{CH}_2\text{-choline}$), 57.2 (d, $J = 7.2$ Hz, C-2), 55.2 (CH, DIPEA), 54.6 ($\text{CH}_3\text{-choline}$ x3), 43.67 (CH_2 DIPEA), 33.4 (C-6), 33.1, 30.80 x4, 30.76, 30.74, 30.47, 30.44, 30.16, 23.7 (CH_2 C-7 to C-17), 14.4 (C-18); ^{31}P NMR (162 MHz, $\text{MeOD-}d_4$) δ -0.37; IR (neat): 3429, 2948, 2846, 1635, 1541, 1461, 1248, 1067, 1030, 928 cm^{-1} ; HRMS calculated for $[\text{C}_{23}\text{H}_{49}\text{N}_2\text{O}_5\text{P} + \text{H}]^+$: 465.3459, found 465.3475.

[5,6,7,8,9- $^{13}\text{C}_5$]-Sphingosine-1-cholinephosphate (19b). $^{13}\text{C}_5$ -Sphingosine acceptor (**9b**) (50 mg, 98 μmol , 1.0 eq) was co-evaporated twice in toluene (5 mL) and then dissolved in anhydrous MeCN (4 mL) under protected atmosphere. 2-Cyanoethoxy-diisopropylamino-2-trimethylammonium-ethoxyphosphine **15** (125 mg, 0.2 mmol, 2.0 eq) was added followed by the addition of tetrazole (0.45 M in MeCN) (0.22 mL, 0.1 mmol, 1.0 eq). The reaction was stirred until TLC showed complete conversion of the sphingosine acceptor (~2 hours). Anhydrous *t*BuOOH (~5.5 M in nonane) (91



μL , 0.5 mmol, 5.0 eq) was added and the reaction was stirred (~ 30 minutes) at room temperature until ^{31}P NMR showed full conversion. DBU (73 μL , 0.5 mmol, 5.0 eq) was added and stirred (~ 1 hour) at room temperature until HPLC-MS showed full removal of 2-cyanoethoxy group. The reaction was concentrated *in vacuo* and coevaporated twice with toluene (5 mL). The crude reaction mixture was then dissolved in methanol (2.5 mL) and sodium methoxide (30% in methanol) (0.26 mL, 2.0 mmol, 20 eq) was added. The reaction was stirred for 3 days at room temperature and the progression of the reaction was followed by HPLC-MS. The reaction was neutralized with Amberlite H^+ resin and the reaction mixture was filtered and concentrated *in vacuo*. The crude reaction mixture was coevaporated in toluene (2.5 mL) and put on ice-bath before addition of DCM (1 mL) and TFA (1 mL). The reaction mixture was stirred for 5 minutes at 0°C . The solution was diluted with toluene (10 mL) and concentrated to about 2 mL *in vacuo*. The coevaporation was repeated two times with toluene (10 mL), before concentration to dryness. The completion of the reaction was confirmed as well purified by HPLC-MS producing [5,6,7,8,9, $^{13}\text{C}_5$]-sphingosine-1-cholinephosphate **19b** (25 mg, 55 μmol , 56%). ^1H NMR (600 MHz, $\text{MeOD-}d_4$) δ 5.89 (dm, 1 H, $J = 151.0$ Hz, H-5), 5.49 (m, 1 H, H-4), 4.30 (m, 3 H, H-3 and $\text{CH}_2\text{-Choline}$), 4.13 (m, 1 H, H-1_a), 4.04 (m, 1 H, H-1_b), 3.66 (m, 2 H, $\text{CH}_2\text{-Choline}$), 3.37 (m, 1 H, H-2), 3.23 (s, 9 H, $\text{CH}_3\text{-Choline}$ x3), 2.11 (dm, 2 H, $J = 126.7$ Hz, H-6), 1.57-1.15 (m, 22 H, H-7 to H-17), 0.90 (t, 3 H, $J = 7.2$ Hz, H-18); ^{13}C NMR (151 MHz, $\text{MeOD-}d_4$) 137.1 (d, $J = 42.6$ Hz, C-5), 128.2 (d, $J = 72.0$ Hz, C-4), 70.7 (d, $J = 5.3$ Hz, C-3), 67.4 (m, $\text{CH}_2\text{-Choline}$), 63.4 (d, $J = 5.1$ Hz, C-1), 60.6 (d, $J = 5.0$ Hz, $\text{CH}_2\text{-Choline}$), 57.2 (d, $J = 7.2$ Hz, C-2), 54.7 ($\text{CH}_3\text{-Choline}$ x3), 33.8 – 32.9 (m, C-6_{sp} and $\text{CH}_2\text{-Sp}$), 30.9 – 29.8 (m, $\text{CH}_2\text{-Sp}$ x10), 23.7 ($\text{CH}_2\text{-Sp}$), 14.4 (C-18_{sp}); ^{31}P NMR (162 MHz, $\text{MeOD-}d_4$) δ -0.51; IR (neat): 3429, 2948, 2846, 1635, 1541, 1461, 1248, 1067, 1030, 928 cm^{-1} ; HRMS Calculated for $[\text{C}_{23}\text{H}_{49}\text{N}_2\text{O}_5\text{P} + \text{H}]^+$: 470.3104, found 465.3475

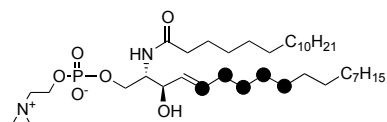
Sphingomyelin (20a) . Sphingosine **19a** (15 mg, 0.032 mmol, 1 eq) was mixed with BSA (0.4 mL) under protected



atmosphere and stirred overnight at room temperature. The mixture was concentrated in high vacuum and the silylated phosphosphingosine was dissolved in dry DCM (0.4 mL) under a protected atmosphere and cooled to 0°C . To the solution was added DIPEA (17 μL , 0.096 mmol, 3.0 eq) followed by palmitoyl

chloride (12 μL , 0.04 mmol, 1.2 eq). The reaction was left stirring, reaching room temperature over 2 h. The reaction mixture was then concentrated *in vacuo* and product was purified by silica column chromatography (9:1 Chloroform/MeOH to 70:27:3 Chloroform:MeOH:H₂O) giving the titeld product **20a** as white solid (14 mg, 0.02 mmol, 61%). $R_f = 0.18$ (70:27:3 Chloroform:MeOH:H₂O); ^1H NMR (600 MHz, $\text{MeOD-}d_4$) δ 5.70 (dt, 1 H, $J = 15.2$, 6.8 Hz, H-5), 5.44 (dd, 1 H, $J = 15.2$, 7.6 Hz, H-4), 4.28 (m, 2 H, $-\text{OCH}_2\text{-choline}$), 4.11 (m, 1 H, H-1_a), 4.04 (t, 1 H, $J = 8.0$ Hz, H-3), 3.99-3.94 (m, 2 H, H-1_b and H-2), 3.64 (m, 2 H, $-\text{CH}_2\text{N}_{\text{choline}}$), 3.30 (s, 9 H, 3x $\text{CH}_3\text{-choline}$), 2.18 (t, 2 H, $J = 7.2$ Hz, H-2*), 2.04 (m, 2 H, H-6), 1.59 (m, 2 H, H-7), 1.42-1.20 (m, 48 H, H-8 to H-17 and H-3* to H-15*), 0.90 (t, 6 H, $J = 6.8$ Hz, H-18 nd H-16*); ^{13}C NMR (150 MHz, $\text{MeOD-}d_4$) δ 175.8 (C=O), 135.0 (C-5), 130.8 (C-4), 76.3 (C-3), 70.7 ($-\text{CH}_2\text{N}_{\text{choline}}$), 63.6 (d, $J = 4.5$ Hz, C-1), 60.6 (d, $J = 4.5$ Hz, $-\text{OCH}_2\text{-choline}$), 57.2 (C-2), 54.7 (3x $\text{CH}_3\text{-choline}$), 37.3, 33.9, 32.9, 30.76, 30.72, 30.69, 30.68, 30.67, 30.65, 30.59, 30.52, 30.39, 30.37, 30.35, 30.31, 27.03, 23.63 (25x CH_2 C-6 to C-17 and C-3* to C15*), 14.4 (C-18 and C-16*); ^{31}P NMR (162 MHz, $\text{MeOD-}d_4$) δ -0.51; IR (neat): 3429, 2915, 2846, 1724, 1633, 1545, 1459, 1250, 1067, 1030, 928 cm^{-1} ; HRMS Calculated for $[\text{C}_{39}\text{H}_{79}\text{N}_2\text{O}_6\text{P} + \text{H}]^+$: 702.5657, found 702.5655.

1-Cholinephosphate-2-N-(hexadecanoyl)-[5,6,7,8,9- $^{13}\text{C}_5$]-sphingosine (20b). Sphingosine **19b** (10 mg, 0.021



mmol, 1 eq) was mixed with BSA (0.3 mL) under protected atmosphere and stirred overnight at room temperature. The mixture was concentrated in high vacuum and the silylated phosphosphingosine was dissolved in dry DCM (0.3 mL) under a protected atmosphere and cooled to 0°C . To the solution was

added DIPEA (12 μL , 0.063 mmol, 3.0 eq) followed by palmitoyl chloride (9 μL , 0.032 mmol, 1.2 eq). The reaction was left stirring, reaching room temperature over 2 h. The reaction mixture was then concentrated *in vacuo* and the product was purified by silica column chromatography (9:1 Chloroform/MeOH to 70:27:3

Chloroform:MeOH:H₂O) giving the titled product **20b** as white solid (10 mg, 0.014 mmol, 66%). *R*_f = 0.18 (70:27:3 Chloroform:MeOH:H₂O); ¹H NMR (600 MHz, CDCl₃) δ 5.68 (dm, 1 H, *J* = 151 MHz, H-5), 5.44 (m, 1 H, H-4), 4.28 (m, 2 H, -OCH₂-choline), 4.12 (m, 1 H, H-1_a), 4.03 (m, 1 H, H-3), 3.98-3.94 (m, 2 H, H-1_b and H-2), 3.65 (m, 2 H, -CH₂N_{choline}), 3.29 (s, 9 H, 3x CH₃-choline), 2.17 (t, 2 H, *J* = 7.2 Hz, H-2*), 2.03 (m, 2 H, H-6), 1.56-1.14 (m, 48 H, H-7 to H-17 and H-3* to H-15*), 0.88 (t, 6 H, *J* = 6.8 Hz, H-18 and H-16*); ¹³C NMR (151 MHz, MeOD-*d*₄) δ 175.6 (C=O), 135.6 (d, *J* = 42.4 Hz, C-5), 128.2 (d, *J* = 72 Hz, C-4), 76.2 (d, *J* = 4.5 Hz, C-3), 70.6 (-CH₂N_{choline}), 63.4 (d, *J* = 4.5 Hz, C-1), 60.3 (d, *J* = 4.5 Hz, -OCH₂-choline), 57.1 (C-2), 54.6 (3x CH₃-choline), 37.1 (C-2*), 33.6-33.0 (m) 30.5-29.8 (m), 26.8 (25x CH₂ C-6 to C-17 and C-3* to C15*), 14.4 (C-18 and C-16*); ³¹P NMR (162 MHz, MeOD-*d*₄) δ -0.51; IR (neat): 3429, 2915, 2846, 1724, 1633, 1545, 1459, 1250, 1067, 1030, 928 cm⁻¹; HRMS Calculated for [C₃₄¹³C₅H₇₉N₂O₆P +H]⁺: 707.5602, found 707.5599.

4.5 References and notes

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Chapter 5

Synthesis of 6-Hydroxysphingosine and Alpha-Hydroxy Ceramide using a Cross-Metathesis Strategy

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5.1 Introduction

(Glyco)sphingolipids are a family of complex lipids found in all mammalian cells. Besides playing important structural roles as membrane components, they are involved in a multitude of intra- and intercellular signaling events and play a role in many (patho)physiological processes.^[1] This structurally diverse class of lipids is composed of a sphingosine base, which can be acylated at the nitrogen with a variety of acyl chains.^[2]

Further structural variation comes from differences in substitution at the primary alcohol group, at which position a large variety of glycans and phosphate groups can be attached. Structural modifications in the sphingosine base are also found. The most recently reported members of the human sphingolipid family, the 6-hydroxyceramides (e. g. **2**, Figure 5.1) were discovered in 1989^[3] and their structure, based on a 6-hydroxysphingosine base (**1**, see Figure 5.1), was fully established in 1994.^[4] These sphingolipids are important constituents of the human skin, especially the stratum corneum (SC), where they play a role in skin barrier pathologies.^[5] Authentic samples of these sphingolipids are valuable to study their role in skin physiology processes. Because they are not commercially available and cannot be obtained from natural sources in pure form and sufficient quantity the development of synthetic routes to access these molecules is important.^[6] To date three reports describing the synthesis of the 6-hydroxysphingosine base have appeared.^[7-9] These three syntheses all hinge on the diastereoselective nucleophilic attack of an appropriately protected alkyne lipid to (S)-Garner aldehyde¹⁰ (**3**, See Figure 5.1), using strongly basic conditions and necessitating a subsequent reduction step.

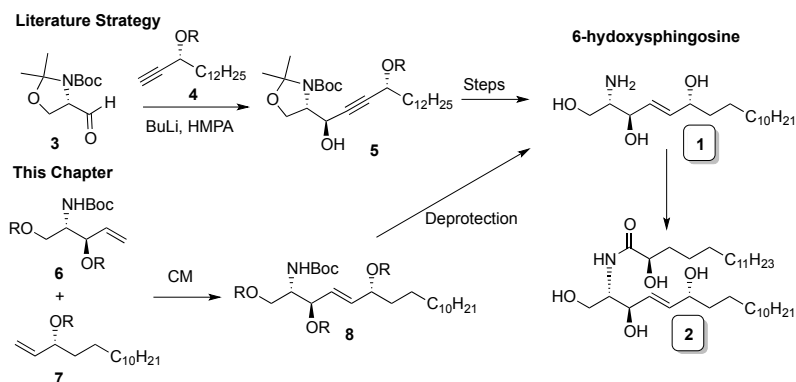
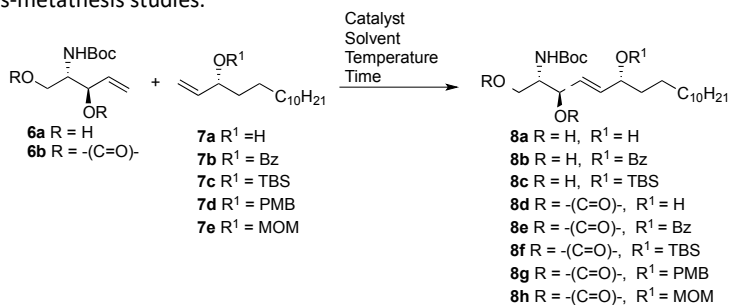


Figure 5.1 Literature strategy and this chapter.

Chapter 2 describes the assembly of (glyco)sphingolipids using a cross-metathesis (CM) strategy as the key step.^[11-14] In this approach an allylic sphingosine head is coupled with a long chain alkene (varying in length and carrying different functionalities^[12] or ¹³C labels^[13]) through the formation of the *E*-double bond. The mild conditions required for this transformation and the broad functional group tolerance make this an attractive strategy and therefore it could be an effective approach to access 6-hydroxysphingosines. To make this strategy successful, difficulties associated with the cross-metathesis of two similar allylic alcohols (Type II or III CM coupling partners)^[15] had to be overcome. This Chapter describes that CM can be used as a key step in the synthesis of 6-hydroxysphingosine **1** (See Figure 5.1) and, from there, in the synthesis of ceramide **2**.

95:5). The α,β -unsaturated ester **10** was then reduced to the allylic alcohol **11** with di-*iso*-butylaluminium hydride (DIBAL-H) to set the stage for Sharpless asymmetric epoxidation, which was used to introduce chirality in the molecule. After substantial optimization of this reaction, optimal conditions were found in the use of 13.5 mol% $\text{Ti}(\text{O}i\text{Pr})_4$, 17.5 mol% D-(-)-di-ethyltartrate (D-(-)-DET), 2.2 equivalents of *tert*-butylhydroperoxide (*t*BuOOH), and molecular sieves 4Å (1 gram/mol) in dichloromethane at -19 °C. This delivered the chiral epoxide **13** in 79% yield and 89% *ee* (determined from tosylate **14**). Installation of a tosylate function at the primary alcohol allowed for the tellurium mediated reductive elimination reaction. To this end an aqueous solution of Na_2Te was generated from tellurium and rongalite ($\text{HOCH}_2\text{SO}_2\text{Na}$) in NaOH (aq).^[16] Addition of **14** to this solution led to the nucleophilic displacement of the primary tosylate by tellurium ion, after which the ensuing epoxide ring opening generates an epitelluride ring that collapses upon exposure to air to give the “transposed” secondary allylic alcohol **7a**. With both allylic alcohols in hand the stage was set for the crucial CM reaction. Generally for a productive CM event, two reaction partners of differing reactivity are required. Grubbs and co-workers^[14] have divided alkenes in four categories based on their ability to form homodimers in CM reactions. Type I alkenes undergo rapid homodimerization and these can participate in an ensuing CM event. Type II alkenes undergo slow homodimerization leading to homodimers that can participate in CM to a limited extent. Type III alkenes do not form homodimers but they are able to react with type I and II homodimers in a CM. Type IV alkenes are essentially “spectators to cross-metathesis” as they do not display any activity towards the catalyst. For a selective CM, the reaction partners are preferably from different types. The two allylic alcohols at hand are classified as type II CM partners, making the desired CM reaction a challenging process. To discriminate between the reactivity of the alkenes the decision was made to protect the long chain alcohol **7a** (generating a type III CM partner) and mask the hydroxyl group as a benzoyl ester (**7b**), a *tert*-butyldimethylsilyl ether (**7c**), a *para*-methoxybenzyl (PMB) ether (**7d**) or methoxymethyl (MOM) ether (**7e**). The results of the CM reactions are summarized in Table 1. The first attempt, involving diol **6a** and alcohol **7a**, provided the desired CM **8a** product in 23% yield (entry 1). Raising the temperature of this reaction did not lead to an improved reaction (entry 2). Next a CM was attempted with the more electron-poor benzoylated allylic alcohol **7b** in combination with diol **6a**, but this CM was unproductive (entry 3). Similarly the use of silylated CM partner **7c** was to no avail (entry 4). Because modulation of the reactivity of the long chain allylic alcohol proved

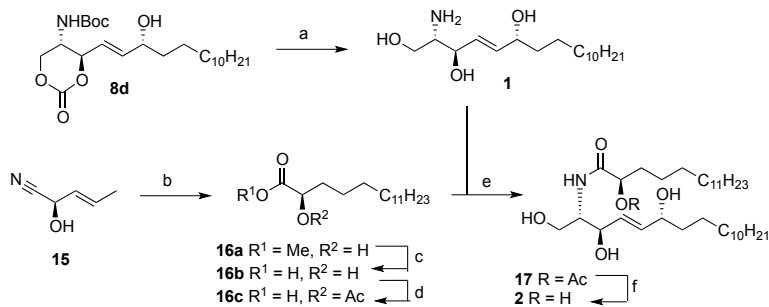
Table 5.1 Cross-metathesis studies.

| entry | 6/7 | Catalyst ^a | solvent | Temperature (°C) | time (h) | Product (yield) ^b |
|-------|--------------|-----------------------|-------------------|---------------------|-------------|---------------------------------|
| 2 | 6a/7a | Grubbs II | DCE | 80 | 48 | 8a (-) |
| 3 | 6a/7b | Grubbs II | DCM | 40 | 48 | 8b (-) |
| 4 | 6a/7c | Grubbs II | DCM | 40 | 48 | 8c (-) |
| 5 | 6b/7a | Grubbs II | DCM | 40 | 48 | 8d (48%) |
| 6 | 6b/7b | Grubbs II | DCM | 40 | 48 | 8e (-) ^c |
| 7 | 6b/7c | Grubbs II | DCM | 40 | 48 | 8f (-) ^c |
| 8 | 6b/7d | Grubbs II | DCM | 40 | 48 | 8g (37%) ^c |
| 9 | 6b/7e | Grubbs II | DCM | 40 | 66 | 8h (43%) ^c |
| 10 | 6b/7a | Grubbs II | DCE | 80 | 48 | 8d (-) |
| 11 | 6b/7a | Hoveyda- Grubbs | DCM | 40 | 70 | 8d (48%) |
| 12 | 6b/7a | Grubbs II + CuI | Tol. | 40 | 48 | 8d (78%) |
| 13 | 6b/7a | Grubbs II + CuI | Et ₂ O | r.t. | 48 | 8d (83%) |

^aReaction conditions: Ratio **6:7** = 3:1; 20 mol % catalyst; 30 mol % (CuI). ^bYields denote isolated yields after column chromatography. ^cUnreacted cyclic carbonate **6b** as well the protected allylic olefin (**7b**, **7c**, **7d** and **7e**) could be recovered.

unsuccessful, it was decided to protect diol **6** as the cyclic carbonate and to investigate how the resulting **6b** would behave in CM events. The cyclic carbonate group is both strongly electron withdrawing, changing the electronic properties of the alkene, and ties back the functionalities of the groups attached to the alkene, making it more accessible. When the allylic carbonate **6b** and alcohol **7a** were combined in a CM event the desired *E*-alkene **8d** was obtained in an increased yield (48%, entry 5). The use of benzoylated and silylated CM partners **7b** and **7c** again led to unproductive CM reactions (entries 6 and 7). The use of PMB and MOM protected allylic alcohols **7d** and **7e** did deliver the desired CM products **8g** and **8h**, respectively, but in a lower yield than obtained for **8e** (entries 8 and 9). Having established that the most productive CM reaction occurs between carbonate **6b** and alcohol **7a**, this reaction was further optimized. Raising the temperature led to decomposition of the cyclic carbonate and therefore no product was obtained (entry 10). Also different catalytic systems were explored. As the use of the Grubbs-Hoveyda catalyst led to an identical result as compared to the Grubbs II catalyzed CM reaction (entry 11), the next step was to explore the impact of additives on the reaction. As described by Voigtritter *et al.*^[17], copper iodide (CuI) can be used to generate a more stable catalyst (due to the iodide), while making the system more reactive because the copper sequesters the phosphine ligands. As shown in entries 12 and 13, the use of this additive proved very successful and alkene **6b** and allylic alcohol **7a** could be fused to provide the desired *E*-alkene **8d** in good yield.

Scheme 5.2 Synthesis of 6-hydroxysphingosine **1** and α -hydroxy ceramide **2**.



Reagents and conditions: (a) LiOH, THF/H₂O (3:1), 0 °C, 3 h; (ii) TFA, DCM, 0 °C, 90 min, 80% over two steps; (b) HCl (g), MeOH, Et₂O, 0 °C to -20 °C, 20 h, then H₂O, 48%; (iii) 1-tetradecene, Grubbs 2nd generation catalyst, AcOH, DCM, 50 °C, 60 h, 73%; (iii) H₂ (g), Pd/C, EtOAc, r.t., 20 h, 91%; (c) LiOH.H₂O, THF:MeOH:H₂O (2:2:1), r.t., 20 h, 99%; (d) Ac₂O, pyridine, DCM, r.t., 20 h, 97%; (e) EEDQ, EtOH, 50 °C, 20 h, 66%; (f) K₂CO₃, DCM/MeOH (4:1), r.t., 2 h, 88%.

Having successfully constructed the 6-hydroxysphingosine backbone, the base was globally deprotected by saponification of the carbonate group and ensuing removal of the Boc protective group using dilute acid at low temperature (0 °C) to give α -hydroxy ceramide **1**, as depicted in Scheme 5.2. The use of more forceful acidic conditions led to

complex reaction mixtures, presumably as a result of acid catalyzed allylic substitution reactions. The synthesis of the 6-hydroxysphingosine based ceramide **2**, featuring an α -hydroxyl side chain was finally accomplished as follows. First α -hydroxy fatty acid **16a** was generated from optically pure cyanohydrin **15**, obtained from crotonaldehyde by the action of almond hydroxynitrilase.^[18] The cyanohydrin was transformed into the corresponding methyl ester using a Pinner reaction.^[19] Next a CM reaction of the alkene with 1-tetradecene gave the unsaturated long chain lipid that was reduced and saponified to give the fatty acid **16b**. To condense the α -hydroxy fatty acid with the 6-hydroxysphingosine, the α -hydroxy group was first masked with an acetyl group. Activation of acid **16c** with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)^[20] and ensuing condensation then gave acylated ceramide **17**. Final saponification of the acetyl ester completed the synthesis of the target 6-hydroxyceramide **2**.

5.3 Conclusion

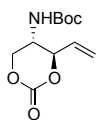
In conclusion, this Chapter presents a new synthetic route for 6-hydroxysphingosine and its alpha-hydroxy-ceramide counterpart, employing a cross-metathesis (CM) strategy. The crucial CM reaction on which the synthesis hinges unites two similar allylic alcohol alkenes. The use of a cyclic carbonate group to protect the diol system, present in the sphingosine CM partner, with a cyclic carbonate functionality serves two purposes. Firstly, it makes the double bond less electron rich, discriminating it from its designated CM partner, the long chain allylic alcohol. Secondly, it ties back the functional groups on the olefin, making the alkene sterically most accessible for the catalyst and the CM event. In combination with an activated catalyst system (the Grubbs II – CuI reagent pair) this led to an efficient CM connection of the sphingosine head and tail alkenes. The mild conditions required for this connection in conjunction with the straightforward deprotection scheme (mild base followed by mild acid) make the approach versatile and amendable to the use of a variety of CM coupling partners to generate labeled and tagged 6-hydroxysphingosine derived probes for future biochemical studies.^[13,21]

5.4 Experimental section

General Remarks. Commercially available reagents and solvents were used as received. DCM and THF were dried and distilled by standard procedures. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out with Silica gel 60 (40–63 μ m mesh). IR spectra are reported in cm^{-1} . Optical rotations were measured with an automatic polarimeter (sodium D-line, λ = 589 nm). The enantiomeric purity was determined by HPLC analysis using an OD column (hexane/isopropyl alcohol (98:2), 1 mL/min, UV 254 nm). NMR spectra were recorded on a 400 MHz or 850 MHz spectrometer. Chemical shifts are reported as δ values (ppm), and were referenced to tetramethylsilane (δ = 0.00 ppm) directly in CDCl_3 , or using the residual solvent peak (D_2O). High-resolution mass spectra were recorded on a LTQ-Orbitrap (Thermo Finnigan) mass spectrometer equipped with an electrospray ion source in positive mode or on a Synapt G2-Si MALDI-TOF mass spectrometer equipped with a 355-nm laser. Samples (1 mL, 100 mM in CHCl_3) were spotted on the MALDI-plate, followed by aqueous silver benzoate (0.5

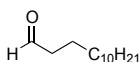
mL, 10 mM), drying and applying the matrix (2,5-dihydroxybenzoic acid, 0.5 mL, 0.5 M in methanol). A laser frequency of 1000 Hz (power set at 60%) was used.

tert-Butyl ((4R,5S)-2-oxo-4-vinyl-1,3-dioxan-5-yl)carbamate (6b). Allylic alcohol **6a** (3.03 g, 13.95 mmol, 1 eq)



was dissolved in anhydrous THF (400 mL) under an argon atmosphere. *N,N'*-Disuccinimidyl carbonate (8.93 g, 34.88 mmol, 2.5 eq) and DMAP (4.26 g, 34.88 mmol, 2.5 eq) were added and then left stirring for 20 hours at room. The reaction mixture was concentrated *in vacuo* and purified with silica gel chromatography (30% EtOAc in pentane) giving Cyclic carbonate **6b** (2.57 g, 10.56 mmol, 76%) as a thick, colorless oil. $R_f = 0.6$ (50% EtOAc in pentane); $[\alpha]_D = +46.0$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.92 (ddd, 1 H, $J = 16.8, 10.8, 4.4$ Hz, H-4), 5.58 (d, 1 H, $J = 7.2$ Hz, -NH), 5.50 (dd, 1 H, $J = 16.8, 1.6$ Hz, H-5_a), 5.47 (dd, 1 H, $J = 10.8, 1.6$ Hz, H-5_b), 5.02 (bs, 1 H, H-3), 4.54 (dd, 1 H, $J = 11.2, 2.4$ Hz, H-1_a), 4.31 (bd, 1 H, $J = 11.2$ Hz, H-1_b), 3.97 (m, 1 H, H-2), 1.46 (s, 9 H, $\text{CH}_3\text{-Boc}$); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 155.25 (C=O_{Boc}), 147.67 (C=O_{Carbonate}), 132.50 (C-4), 119.68 (C-5), 81.95 (C-3), 80.87 (C_{q-Boc}), 68.15 (C-1), 45.84 (C-2), 28.34 ($\text{CH}_3\text{-Boc}$); IR (neat): 2978, 2932, 1751, 1701, 1165 cm^{-1} ; HRMS for calculated $[\text{C}_{11}\text{H}_{18}\text{NO}_5 + \text{H}]^+$; 244.11795, found 244.11835.

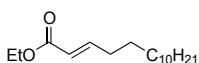
Tridecanal (10). Tridecanoic acid **9** (7.50 g, 35.0 mmol, 1 eq) was dissolved in anhydrous DCM (110 mL) under an



argon atmosphere. The solution was cooled to 0°C before adding oxalyl chloride (2 M in DCM, 35 mL, 70 mmol, 2 eq) and DMF (3 drops, cat.). The mixture was stirred for 4 to 5 hours at room temperature. Once gas formation subsided, the reaction mixture was concentrated *in vacuo* and the crude acyl chloride was immediately dissolved in anhydrous DCM (110 mL) under an argon atmosphere. The solution was cooled to -78°C before slowly addition of distilled *N,O*-dimethylhydroxylamine (6.42 mL, 87.5 mmol, 2.5 eq). After 30 minutes the reaction mixture was allowed to reach room temperature and stirred for 20 hours. The reaction mixture was filtered, concentration *in vacuo*, and purified with silica gel chromatography (5% to 10% EtOAc in pentane) giving *N*-methoxy-*N*-methyltridecanamide (8.94 g, 34.74 mmol, 99%) as an oil. $R_f = 0.3$ (10% EtOAc in pentane); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 3.68 (s, 3 H, -OCH₃), 3.18 (s, 3 H, -NCH₃), 2.41 (t, 2 H, $J = 7.6$ Hz, H-1), 1.63 (p, 2 H, $J = 7.6$ Hz, H-2), 1.31 - 1.26 (m, 18 H, H-3 to H-11), 0.88 (t, 3 H, $J = 6.8$ Hz, H-12); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 175.03 (C=O_{Weinreb}), 61.31 (-OCH₃), 32.30 (-NCH₃), 32.04 (C-1, CH₂), 29.79, 29.76 (x2), 29.64, 29.59, 29.56, 29.48 (CH₂ x 7), 24.79 (C-2), 22.82 (CH₂), 14.25 (C-12); IR (neat): 2922, 2853, 1668 cm^{-1} ; HRMS calculated for $[\text{C}_{15}\text{H}_{32}\text{NO}_2 + \text{H}]$; 258.24276; found 258.24257.

N-methoxy-*N*-methyltridecanamide (2.59 g, 10.05 mmol, 1 eq) was dissolved in anhydrous THF (25 mL) under an argon atmosphere. The solution was cooled to -60 °C followed by addition of diisobutylaluminum hydride (1 M in THF, 12 mL, 12 mmol, 1.2 eq). The reaction mixture was stirred for 3 hours at -60 °C. The reaction was quenched by adding Rochelle salt solution (sat., 20 mL) at -60 °C. The mixture was then allowed to reach room temperature before extracting with EtOAc (2x 150 mL). The combined organic layers were washed with brine, dried over MgSO_4 , filtrated and concentrated *in vacuo*. The crude tridecanal **10** was collected as an oil, which was used for the next reaction without further purification. $R_f = 0.7$ (5% EtOAc in pentane); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 9.76 (t, 1 H, $J = 1.6$ Hz, -CHO), 2.42 (dt, 2 H, $J = 7.2, 1.6$ Hz, H-1), 1.63 (p, 2 H, $J = 7.2$ Hz, H-2), 1.30 - 1.26 (m, 18 H, H-3 to H-11), 0.88 (t, 3 H, $J = 6.8$ Hz, H-12); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 202.96 (C=O_{aldehyde}), 44.03 (C-1), 32.03, 29.76, 29.74, 29.70, 29.55, 29.48, 29.47, 29.28, 22.80, 22.19 (CH₂ x 10), 14.22 (C-12); IR (neat): 3024, 2984, 2941, 1732, 1236 cm^{-1} .

Ethyl (E)-pentadec-2-enoate (11). Diisopropyl (ethoxycarbonylmethyl) phosphonate (3.53 g, 14 mmol, 1.4 eq)



was dissolved in anhydrous THF (40 mL) under an argon atmosphere. *n*-Butyllithium (2.5 M in hexanes, 5 mL, 12.5 mmol, 1.25 eq) was added and stirred for 15 minutes at room temperature followed by addition of a solution of crude tridecanal **10** in anhydrous THF (15 mL). The reaction mixture was stirred for 20 hours. The reaction mixture was diluted with H_2O (100 mL) and extracted with Et_2O (3x 100 mL). The combined organic layers were washed with brine, dried over

MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (0-2% EtOAc in pentane). The α,β -unsaturated ester **11** (2.08 g, 7.73 mmol, 77% over 2 steps, $\geq 98\%$ E) was collected as a light yellow oil. $R_f = 0.5$ (2% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 6.97 (dt, 1 H, $J = 15.6, 6.8$ Hz, H-4), 5.81 (dt, 1 H, $J = 15.6, 1.6$ Hz, H-3), 4.18 (q, 2 H, $J = 7.2$ Hz, H-2), 2.19 (dq, 2 H, $J = 7.2, 1.2$ Hz, H-5), 1.45 (m, 2 H, H-6), 1.32 – 1.26 (m, 21 H, H-1 and H-7 to H-15), 0.88 (t, 3 H, $J = 6.8$ Hz, H-16); ¹³C NMR (101 MHz, CDCl₃) δ 166.89 (C=O), 149.60 (C-4), 121.30 (C-3), 60.21 (C-2), 32.32 (C-5), 32.04, 29.78, 29.76 (x2), 29.65, 29.52, 29.48, 29.28 (CH₂ x 8), 28.14 (C-6), 22.81 (CH₂), 14.39 (C-1), 14.23 (C-16); IR (neat): 2922, 2853, 1722, 1655, 1179 cm⁻¹; HRMS calculated for [C₁₇H₃₃O₂ + H]⁺: 269.2475, found 269.2475. Spectroscopic data was identical to literature.^[7]

(E)-Pentadec-2-en-1-ol (12). Ethyl (E)-pentadec-2-enoate **11** (2.39 g, 8.89 mmol, 1 eq) was dissolved in anhydrous THF (45 mL) under an argon atmosphere. The solution was cooled to -78°C followed by addition of diisobutylaluminum hydride (1M in THF, 26.7 mL, 26.7 mmol, 3 eq). The reaction mixture was stirred for 3 hours, slowly warming up to -60°C. The reaction was quenched with NH₄Cl solution (sat.) at -60°C. The mixture was then allowed to warm to room temperature before adding HCl (5%, 100 mL). The mixture was extracted with Et₂O (3x 100 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (2.5-5% EtOAc in pentane). Allylic alcohol **12** (1.89 g, 8.34 mmol, 94%) was collected as a colorless oil that slowly crystallized into a white solid. $R_f = 0.3$ (5% EtOAc in pentane): ¹H NMR (400 MHz, CDCl₃) δ 5.73 – 5.59 (m, 2 H, H-2 & H-3), 4.08 (d, 2 H, $J = 6.0$ Hz, H-1), 2.04 (m, 2 H, H-4), 1.50 (bs, 1 H, -OH), 1.37 (m, 2 H, H-5), 1.32 – 1.26 (m, 18 H, H-6 to H-14), 0.88 (t, 3 H, $J = 6.8$ Hz, H-15); ¹³C NMR (101 MHz, CDCl₃) δ 133.75, 128.90 (C-2, C-3), 63.98 (C-1), 32.36 (C-4), 32.06, 29.83, 29.81, 29.79, 29.76, 29.65, 29.50, 29.34, 29.28, 22.83 (CH₂ x 10), 14.26 (C-15); IR (neat): 3292, 2955, 2918, 2849, 1672, 1462 cm⁻¹. Spectroscopic data was identical to literature.^[7]

((2R,3R)-3-dodecyloxiran-2-yl)methanol (13). Activated, powdered molecular sieves (~5 g, 4Å) were added to a flame dried flask with freshly distilled, anhydrous DCM (15 mL) under an argon atmosphere. The suspension was stirred for 10 minutes at room temperature, completely drying DCM in the process. (-)-Diethyl D tartrate (0.15 mL, 0.86 mmol, 0.17 eq) and titanium (IV) isopropoxide (0.2 mL, 0.69 mmol, 0.14 eq) were added to this solution at -19°C. Allylic alcohol **12** (1.13 g, 5.00 mmol, 1 eq) was co-evaporated with toluene (2x) and dissolved in freshly distilled, anhydrous DCM (10 mL). After stirring the titanium-tartrate solution for 30 minutes, the solution of allylic alcohol **12** was added followed by addition of *tert*-butyl hydroperoxide (~5.5 M in decane, 2 mL, 11.0 mmol, 2.2 eq). The reaction mixture was stirred for 5 hours at -19 °C. The reaction was quenched by adding H₂O (20 mL) at -19 °C. After warming up to room temperature, NaOH (30% in brine, 2.5 mL) was added to hydrolysis of (-)-Diethyl D tartrate. The mixture was stirred for 15 minutes, followed by extraction with DCM (3x 30 mL). The combined organic layers were dried over MgSO₄, filtrated and concentrated *in vacuo*. Remaining *tert*-butyl hydroperoxide was removed by co-evaporation with toluene. The crude product was purified with silica gel chromatography (10-15% EtOAc in pentane). Epoxide **13** (0.92 g, 3.80 mmol, 76%, e.e. = 89% (determined from tosylate **14**) was collected as a white solid. The analytical sample was recrystallized from petroleum ether (40-60%). $R_f = 0.3$ (20% EtOAc in pentane); $[\alpha]_D = +23.4$ ($c = 1.0$, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 3.92 (ddd, 1 H, $J = 12.4, 5.2, 2.4$ Hz, H-1a), 3.63 (ddd, 1 H, $J = 12.0, 7.2, 4.4$ Hz, H-1b), 2.96 (m, 1 H, H-2), 2.92 (m, 1 H, H-3), 1.66 (m, 1 H, -OH), 1.56 (m, 2 H, H-4), 1.44 (m, 2 H, H-5), 1.35 – 1.26 (m, 18 H, H-6 to H-14), 0.88 (t, 3 H, $J = 6.8$ Hz, H-15); ¹³C NMR (101 MHz, CDCl₃) δ 61.82 (C-1), 58.51 (C-3), 56.13 (C-2), 32.07 (CH₂), 31.71 (C-4), 29.81, 29.78 (x2), 29.70, 29.68, 29.55, 29.50 (CH₂ x 7), 26.10 (C-5), 22.84 (CH₂), 14.28 (C-15); IR (neat): 3252, 2953, 2916, 2870, 2847, 1458, 1248, 868 cm⁻¹; HRMS calculated for [C₁₅H₃₁O₂ + H]⁺: 243.2319, found 243.2321. Spectroscopic data was identical to literature.^[8,22]

((2R,3R)-3-dodecyloxiran-2-yl)methyl 4-methylbenzenesulfonate (14). Epoxide **13** (716 mg, 2.95 mmol, 1 eq)

was dissolved in anhydrous DCM (30 mL) under an argon atmosphere. The solution was cooled to 0 °C followed by addition of *p*-toluenesulfonyl chloride (1.13 g, 5.91 mmol, 2 eq), DMAP (25 mg, 0.2 mmol, cat.) and triethylamine (1.24 mL, 8.86 mmol, 3 eq). The reaction mixture was stirred for 20 hours at room temperature. The reaction was quenched with H₂O (30 mL). The aqueous layer was extracted with DCM (3x 30 mL). The combined organic layers were washed with H₂O (2x), dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (2-5% EtOAc in pentane). Tosylate **14** (1.12 g, 2.82 mmol, 96%) was collected as colorless oil that slowly crystallized into a white solid. *R*_f = 0.3 (5% EtOAc in pentane); [α]_D²⁰ = +24.0° (*c* = 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.00 (ad, 2 H, *J* = 8.4 Hz, H-17), 7.35 (d, 2 H, *J* = 8.0 Hz, H-18), 4.18 (dd, 1 H, *J* = 11.2, 4.0 Hz, H-1_a), 3.97 (dd, 1 H, *J* = 11.2, 6.0 Hz, H-1_b), 2.95 (ddd, 1 H, *J* = 6.0, 4.0, 2.0 Hz, H-2), 2.78 (td, 1 H, *J* = 5.6, 2.0 Hz, H-3), 2.45 (s, 3 H, H-20), 1.51 (m, 2 H, H-4), 1.36 (m, 2 H, H-5), 1.32 – 1.25 (m, 18 H, H-6 to H-14), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15); ¹³C NMR (101 MHz, CDCl₃) δ 145.18 (C-16), 132.86 (C-19), 130.04 (C-18), 128.10 (C-17), 70.34 (C-1), 56.95 (C-3), 54.66 (C-2), 32.05 (CH₂), 31.43 (C-4), 29.79, 29.77, 29.76, 29.65, 29.61, 29.49, 29.43 (CH₂ x 7), 25.87 (C-5), 22.83 (CH₂), 21.81 (C-20), 14.27 (C-15); IR (neat): 2953, 2916, 2870, 2849, 1599, 1364, 1177, 829, 808 cm⁻¹; HRMS calculated for [C₂₂H₃₇O₄S + H]⁺: 397.2407, found 397.2404.

(R)-pentadec-1-en-3-ol (7a). Sodium hydroxymethanesulfinate hydrate (6.50 g, 55.0 mmol, 7.3 eq) was dissolved

in NaOH (1M, 150 mL). Argon was purged through the solution before addition of tellurium (powder, -200 mesh, 1.92 g, 15.1 mmol, 2 eq). The reaction mixture was stirred for 2 hours at 50 °C. The purple solution of tellurides was cooled to 0°C followed by slowly addition of a solution of Tosylate **14** (2.99 g, 7.53 mmol, 1 eq) in THF (75 mL). The reaction was stirred for 20 hours at room temperature. The reaction was quenched by bubbling air through the solution. The crude reaction mixture was then filtrated over a pad of Celite and concentrated *in vacuo*. The aqueous layer was extracted with Et₂O (2x 150 mL). The combined organic layers were washed with H₂O₂ (3%, 75 mL), sodium thiosulfate (10%) and brine before being dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (5-10% Et₂O in pentane). Allylic alcohol **7a** (1.54 g, 6.78 mmol, 90%) was collected as a white solid. *R*_f = 0.4 (20% Et₂O in pentane); [α]_D²⁰ = -7.0° (*c* = 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.87 (ddd, 1 H, *J* = 16.8, 10.4, 6.4 Hz, H-2), 5.22 (dt, 1 H, *J* = 17.2, 1.2 Hz, H-1_a), 5.10 (dt, 1 H, *J* = 10.4, 1.2 Hz, H-1_b), 4.10 (m, 1 H, H-3), 1.69 (bs, 1 H, -OH), 1.52 (m, 2 H, H-4), 1.43 – 1.26 (m, 20 H, H-5 to H-14), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15); ¹³C NMR (101 MHz, CDCl₃) δ 141.45 (C-2), 114.67 (C-1), 73.45 (C-3), 37.19 (C-4), 32.07, 29.82, 29.80, 29.74, 29.70, 29.51, 25.48, 22.84 (CH₂ x 8), 14.28 (C-15); IR (neat): 3348, 2918, 2853, 1643, 1466 cm⁻¹. Spectroscopic data was identical to literature.^[22]

(R)-pentadec-1-en-3-yl benzoate (7b). Allylic alcohol **7a** (325 mg, 1.44 mmol, 1 eq) was dissolved in anhydrous

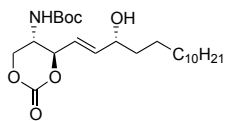
DCM (17 mL) under an argon atmosphere. Benzoic anhydride (974 mg, 4.31 mmol, 3 eq), triethylamine (0.80 mL, 5.74 mmol, 4 eq) and DMAP (17 mg, 0.14 mmol, cat.) were added and the reaction mixture was stirred under reflux for 20 hours. The reaction was quenched by adding NH₄Cl (sat., 25 mL). The aqueous layer was extracted with Et₂O (3x 30 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (0-2% EtOAc in pentane). The benzoylated product **7b** (424 mg, 1.28 mmol, 89%) was collected as an oil. *R*_f = 0.8 (10% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 8.07 (m, 2 H, H-17), 7.55 (m, 1 H, H-19), 7.44 (m, 2 H, H-18), 5.89 (ddd, 1 H, *J* = 17.2, 10.4, 6.4 Hz, H-2), 5.49 (m, 1 H, H-3), 5.32 (dt, 1 H, *J* = 17.2, 1.2 Hz, H-1_a), 5.20 (dt, 1 H, *J* = 10.4, 1.2 Hz, H-1_b), 1.75 (m, 2 H, H-4), 1.46 – 1.25 (m, 20 H, H-5 to H-14), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15); ¹³C NMR (101 MHz, CDCl₃) δ 165.97 (C=O), 136.75 (C-2), 132.95 (C-19), 130.72 (C-16), 129.70 (C-17), 128.44 (C-18), 166.66 (C-1), 75.50 (C-3), 34.47 (C-4), 32.05, 29.80, 29.77 (x2), 29.70, 29.64, 29.54, 29.49, 25.23, 22.83 (CH₂ x 10), 14.27 (C-15); IR (neat): 3088, 3030, 2922, 2853, 1719, 1267 cm⁻¹; HRMS (MALDI-TOF) calculated for [C₂₂H₃₄O₂ + Ag]⁺ 437.1610, found 437.1602.

(R)-tert-butylidimethyl(pentadec-1-en-3-yloxy)silane (7c). Allylic alcohol **7a** (259 mg, 1.14 mmol, 1 eq) was dissolved in anhydrous DMF (5 mL) under an argon atmosphere. DMAP (6 mg, 0.05 mmol, cat.) was added and the solution was cooled to 0 °C followed by addition of *tert*-Butyldimethylsilyl chloride (175 mg, 1.16 mmol, 1.02 eq) and triethylamine (0.18 mL, 1.29 mmol, 1.1 eq). The reaction mixture was stirred for 20 hours at room temperature. The reaction was quenched with H₂O (>50 mL) and extracted with Et₂O (3x 50 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (0% to 2% EtOAc in pentane). The silylated product **7c** was collected as a colorless oil (265 mg, 0.78 mmol, 68%). *R*_f = 0.4 (pentane); ¹H NMR (400 MHz, CDCl₃) δ 5.79 (ddd, 1 H, *J* = 16.8, 10.4, 6.0 Hz, H-2), 5.12 (dt, 1 H, *J* = 17.2, 1.6 Hz, H-1_a), 5.01 (dt, 1 H, *J* = 10.4, 1.6 Hz, H-1_b), 4.07 (m, 1 H, H-3), 1.45 (m, 2 H, H-4), 1.37 – 1.26 (m, 20 H, H-5 to H-14), 0.90 (s, 9 H, CH₃-*t*Bu), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15), 0.05 (s, 3 H, CH₃-Si), 0.03 (s, 3 H, CH₃-Si); ¹³C MR (101 MHz, CDCl₃) δ 142.12 (C-2), 113.48 (C-1), 74.06 (C-3), 38.29 (C-4), 32.10, 29.85, 29.83, 29.82, 29.80, 29.79 (x2), 29.53 (CH₂ x 8), 26.06 (CH₃-Si-*t*Bu), 25.37, 22.86 (CH₂ x 2), 18.44 (C_q-Si), 14.29 (C-15), -4.21 (CH₃-Si), -4.66 (CH₃-Si); IR (neat): 2955, 2924, 2853, 1252, 1080, 814 cm⁻¹; HRMS (MALDI-TOF) calculated for [C₂₁H₄₄OSi + Ag]⁺: 447.2212, found 447.2229.

(R)-1-methoxy-4-((pentadec-1-en-3-yloxy)methyl)benzene (7d). Allylic alcohol **7a** (232 mg, 1.02 mmol, 1 eq) was dissolved in anhydrous DMF (5 mL) under an argon atmosphere. The solution was cooled to 0°C, followed by addition of sodium hydride (60% in mineral oil, 48 mg, 2.0 mmol, 2 eq). After stirring for 15 minutes, 4-methoxybenzyl chloride (0.27 mL, 2.0 mmol, 2 eq) was slowly added to the reaction mixture. The solution was stirred for 20 hours at room temperature. The reaction was quenched by adding NH₄Cl (sat., 20 mL) drop wise. After quenching, H₂O (>50 mL) was added and extracted with Et₂O (2x 100 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (0% to 1% Et₂O in pentane). The PMB-protected product **7d** (309 mg, 0.89 mmol, 87%) was collected as a light yellow oil. *R*_f = 0.2 (1% Et₂O in pentane); [α]_D = +19.0° (*c* = 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.25 (ad, 2 H, *J* = 8.8 Hz, H-18), 6.87 (ad, 2 H, *J* = 8.8 Hz, H-19), 5.72 (ddd, 1 H, *J* = 18.4, 10.4, 8.0 Hz, H-2), 5.21 (dm, 1 H, H-1_a), 5.18 (dm, 1 H, H-1_b), 4.52 (d, 1 H, *J* = 11.6 Hz, H-16_a), 4.28 (d, 1 H, *J* = 11.6 Hz, H-16_b), 3.80 (s, 3 H, H-21), 3.69 (m, 1 H, H-3), 1.63 (m, 1 H, H-4_a), 1.46 (m, 1 H, H-4_b), 1.40 – 1.25 (m, 20 H, H-5 to H-14), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15); ¹³C NMR (101 MHz, CDCl₃) δ 159.14 (C_q-PMB), 139.50 (C-2), 131.09 (C_q-PMB), 129.46 (C-18), 117.00 (C-1), 113.85 (C-19), 80.44 (C-3), 69.80 (C-16), 55.42 (C-21), 35.67 (C-4), 32.08, 29.83, 29.81 (x2), 29.78, 29.75, 29.70, 29.52, 25.54, 22.85 (CH₂ x 10), 14.28 (C-15); IR (neat): 2922, 2853, 1612, 1246, 1038 cm⁻¹; HRMS (MALDI-TOF) calculated for [C₁₇H₃₄O₂ + Ag]⁺: 377.1610, found 377.1598.

(R)-3-(methoxymethoxy)pentadec-1-ene (7e). Allylic alcohol **7a** (229 mg, 1.01 mmol, 1 eq) was dissolved in anhydrous DCM (6 mL) under an argon atmosphere. DMAP (16 mg, 0.13 mmol, cat.) was added and the solution was cooled to 0 °C. DIPEA (0.87 mL, 5.0 mmol, 5 eq) and chloromethyl methyl ether (0.34 mL, 4.5 mmol, 4.5 eq) were added. The reaction was stirred for 20 hours at room temperature. The reaction was quenched by adding NH₄Cl (sat., 20 mL). The reaction mixture was diluted with H₂O and extracted with DCM (3 x 50 mL). The combined organic layers were dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (1-20% Toluene in pentane). The MOM-protected product **7e** (256 mg, 0.95 mmol, 94%) was collected as a light yellow oil. *R*_f = 0.2 (20% Toluene in pentane); [α]_D = +50.0° (*c* = 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.66 (ddd, 1 H, *J* = 17.6, 10.4, 7.6 Hz, H-2), 5.19 (dm, 1 H, H-1_a), 5.16 (dm, 1 H, H-1_b), 4.70 (d, 1 H, *J* = 6.8 Hz, H-16_a), 4.53 (d, 1 H, *J* = 6.8 Hz, H-16_b), 3.97 (m, 1 H, H-3), 3.37 (s, 3 H, H-17), 1.61 (m, 1 H, H-4_a), 1.49 (m, 1 H, H-4_b), 1.45 – 1.26 (m, 20 H, H-5 to H-14), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15); ¹³C NMR (101 MHz, CDCl₃) δ 138.69 (C-2), 117.09 (C-1), 93.83 (C-16), 77.52 (C-3), 55.49 (C-17), 35.58 (C-4), 32.07, 29.82, 29.80 (x2), 29.76, 29.75, 29.70, 29.51, 25.53, 22.83 (CH₂ x 10), 14.25 (C-15); IR (neat): 2924, 2853, 1036 cm⁻¹; HRMS (MALDI-TOF) calculated for [C₂₂H₃₄O₂ + Ag]⁺: 437.1610, found 437.1602.

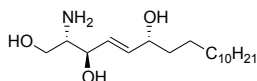
tert-Butyl ((4R,5S)-4-((R,E)-3-hydroxypentadec-1-en-1-yl)-2-oxo-1,3-dioxan-5-yl)carbamate (8d). Allylic alcohol



7a (24 mg, 0.11 mmol, 1 eq) and cyclic carbonate **6b** (73 mg, 0.30 mmol, 3 eq) were combined and co-evaporated with toluene in a 50 mL round bottom flask. The mixture was dissolved in anhydrous Et₂O (0.5 mL) under an argon atmosphere. The solution was stirred briefly before addition of second generation Grubbs Catalyst (17 mg, 0.02 mmol, 0.2 eq) and copper (I) iodide (6 mg, 0.03 mmol, 0.3 eq). The reaction

mixture was stirred at room temperature for 48 hours. The reaction mixture was concentrated *in vacuo*. The crude product was purified with silica gel chromatography (20-30% EtOAc in pentane). The metathesized product **8d** was collected as a brown oil that slowly crystallized (39 mg, 0.088 mmol, 83%). *R_f* = 0.4 (40% EtOAc in pentane); $[\alpha]_D^{25} = +24.4^\circ$ (*c* = 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.96 (dd, 1 H, *J* = 15.6, 4.8 Hz, H-5), 5.78 (dd, 1 H, *J* = 15.2, 4.8 Hz, H-4), 5.53 (d, 1 H, *J* = 7.2 Hz, -NH), 5.00 (m, 1 H, H-3), 4.55 (bd, 1 H, *J* = 9.6 Hz, H-1_a), 4.30 (bd, 1 H, *J* = 10.4 Hz, H-1_b), 4.19 (m, 1 H, H-6), 3.94 (bs, 1 H, H-2), 2.41 (bs, 1 H, -OH), 1.51 (m, 2 H, H-7), 1.46 (s, 9 H, CH₃-Boc), 1.32 – 1.26 (m, 20 H, H-8 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ¹³C NMR (101 MHz, CDCl₃) δ 155.25 (C=O_{Boc}), 147.83 (C=O_{Carbonate}), 139.19 (C-5), 123.77 (C-4), 81.73 (C-3), 80.93 (C_q-Boc), 71.32 (C-6), 68.36 (C-1), 46.20 (C-2), 37.16 (C-7), 32.00, 29.76, 29.74 (x2), 29.71, 29.66, 29.61, 29.44 (CH₂ x 8), 28.37 (CH₃-Boc), 25.44, 22.77 (CH₂ x 2), 14.21 (C-18); IR (neat): 3464, 3352, 2951, 2917, 2850, 1759, 1695, 1190, 1165 cm⁻¹; HRMS calculated for [C₁₉H₃₆NO₄ (M-Boc) +H]⁺: 342.2639, found 342.2641.

6-Hydroxy sphingosine (1). Protected 6-hydroxysphingosine **8d** (35 mg, 0.080 mmol) was dissolved in THF/H₂O



(3:1, 1.5 mL) at room temperature. The solution was cooled to 0°C before addition of lithium hydroxide monohydrate (9 mg, 0.21 mmol, 2.7 eq). The reaction mixture was stirred for 3 hours at 0°C. The reaction mixture was

acidified by addition of Amberlyst. The reaction mixture was filtrated, washed with MeOH/EtOAc and concentrated *in vacuo*. The crude *tert*-Butyl ((2S,3R,6R,E)-1,3,6-trihydroxyoctadec-4-en-2-yl)carbamate was collected as a solid, which was used for the next reaction without further purification. *R_f* = 0.4 (80% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 5.82 (dd, 1 H, *J* = 15.6, 5.2 Hz, H-5), 5.74 (dd, 1 H, *J* = 15.6, 5.2 Hz, H-4), 5.46 (d, 1 H, *J* = 8.4 Hz, -NH), 4.33 (t, 1 H, *J* = 4.6 Hz, H-3), 4.11 (m, 1 H, H-6), 3.88 (dd, 1 H, *J* = 11.2, 3.6 Hz, H-1_a), 3.67 (dd, 1 H, *J* = 11.2, 3.6 Hz, H-1_b), 3.60 (m, 1 H, H-2), 1.50 (m, 2 H, H-7), 1.44 (s, 9 H, CH₃-Boc), 1.28 – 1.26 (m, 20 H, H-8 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ¹³C NMR (101 MHz, CDCl₃) δ 156.55 (C=O_{Boc}), 135.66 (C-5), 129.57 (C-4), 80.09 (C_q-Boc), 73.64 (C-3), 72.03 (C-6), 62.29 (C-1), 55.44 (C-2), 37.35 (C-7), 32.06, 29.83 (x3), 29.80 (x2), 29.76, 29.50 (CH₂ x 8), 28.55 (CH₃-Boc), 25.70, 22.82 (CH₂ x 2), 14.25 (C-18); HRMS calculated for [C₂₃H₄₅NO₅+Na]⁺: 438.3190, found 438.3187.

The crude *tert*-Butyl ((2S,3R,6R,E)-1,3,6-trihydroxyoctadec-4-en-2-yl)carbamate previously described was dissolved in DCM (2.7 mL). The solution was cooled to 0°C before slowly adding TFA (0.3 mL). The solution was stirred for 90 minutes at 0°C. The reaction mixture was diluted with toluene (~20 mL) followed by concentration *in vacuo*. Before complete evaporation of all solvents, the reaction mixture was diluted with toluene 2 more times (2x 20 mL). The crude product was purified with silica gel chromatography (neutralized silica, 5-7.5% MeOH in CHCl₃). 6-Hydroxy sphingosine **1** was collected as a waxy solid (20 mg, 0.063 mmol, 80% over 2 steps). *R_f* = 0.3 (30% MeOH in CHCl₃); $[\alpha]_D^{25} = -9.6^\circ$ (*c* = 0.5, MeOH); ¹H NMR (400 MHz, MeOD-*d*₄) δ 5.86 (dd, 1 H, *J* = 15.6, 6.0 Hz, H-5), 5.67 (dd, 1 H, *J* = 15.6, 6.4 Hz, H-4), 4.37 (t, 1 H, *J* = 5.2 Hz, H-3), 4.09 (q, 1 H, *J* = 6.0 Hz, H-6), 3.80 (dd, 1 H, *J* = 11.6, 4.0 Hz, H-1_a), 3.70 (dd, 1 H, *J* = 11.6, 8.0 Hz, H-1_b), 3.24 (m, 1 H, H-2), 1.51 (m, 2 H, H-7), 1.46 – 1.29 (m, 20 H, H-8 to H-17), 0.90 (t, 3 H, *J* = 6.8 Hz, H-18); ¹³C NMR (101 MHz, MeOD-*d*₄) δ 138.26 (C-5), 128.69 (C-4), 72.52 (C-6), 70.43 (C-3), 59.28 (C-1), 58.30 (C-2), 38.27 (C-7), 33.05, 30.77 (x2), 30.74 (x3), 30.45, 26.53, 23.71 (CH₂ x 9), 14.44 (C-18); IR (neat): 3329, 3096, 2953, 2922, 2853, 1668, 1464, 1456, 1435, 1200, 1186, 1136 cm⁻¹; HRMS calculated [C₁₈H₃₇NO₃ +H]⁺: C₁₈H₃₇NO₃ 316.2846, found 316.2847. Spectroscopic data was identical to literature.^[8]

Methyl (R)-2-hydroxyhexadecanoate (16a). (R,E)-2-hydroxypent-3-enenitrile **15** (1.95 g, 20.10 mmol, 1 eq, ee > 99%) was dissolved in anhydrous Et₂O (25.0 mL) in a flame-dried three necked round bottom flask under an argon atmosphere. Anhydrous MeOH (1.66 mL, 20.98 mmol, 2.0 eq) was added. This solution was purged with dry HCl gas (1.47 g, 40.19 mmol, 2 eq).

The acidified reaction mixture was stored at -20°C for 20 hours under an argon atmosphere. H₂O (10 mL) was added and stirring for 40 minutes. The aqueous layer was extracted with EtOAc (3x 40 mL). The combined organic layers were washed with NaHCO₃ (sat., 40 mL) and brine, dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude product was purified with silica gel chromatography (10% DCM, 10% Et₂O in pentane, isocratic) giving Methyl (R,E)-2-hydroxypent-3-enoate as a yellow oil (1.26 g, 9.66 mmol, 48%). *R*_f = 0.2 (10% DCM, 10% Et₂O in pentane); ¹H NMR (400 MHz, CDCl₃) δ 5.90 (m, 1 H, H-4), 5.53 (m, 1 H, H-3), 4.61 (d, 1 H, *J* = 6.4 Hz, H-2), 3.80 (s, 3 H, -OMe), 3.20 (bs, 1 H, -OH), 1.74 (d, 3 H, *J* = 6.8 Hz, H-5); ¹³C NMR (101 MHz, CDCl₃) δ 174.26 (C-1), 129.92 (C-4), 127.26 (C-3), 71.48 (C-2), 52.80 (-OMe), 17.74 (C-5); IR (neat): 3439, 2922, 2855, 1732, 1445, 1198 cm⁻¹. Spectroscopic data was identical to literature.^[18]

Methyl (R,E)-2-hydroxypent-3-enoate (1.42 g, 10.94 mmol, 1 eq) was dissolved in anhydrous DCM (6 mL) under an argon atmosphere in a 250 mL round bottom flask. 1-Tetradecene (5.55 mL, 21.88 mmol, 2 eq), acetic acid (63 μL, 1.09 mmol, 0.5 eq) and second generation Grubbs Catalyst (93 mg, 0.11 mmol, 0.05 eq) were added to the reaction mixture. The reaction mixture was stirred for 60 hours at 50 °C. Afterwards, the reaction mixture was concentrated *in vacuo*. The crude product was purified with silica gel chromatography (5% Et₂O, 10% DCM to 7.5 % Et₂O, 10% DCM to 10% Et₂O, 10% DCM in pentane). The metathesized product Methyl (R,E)-2-hydroxyhexadec-3-enoate was collected as a brown oil (2.27 g, 7.99 mmol, 73%). *R*_f = 0.2 (10% Et₂O, 10% DCM in pentane); ¹H NMR (400 MHz, CDCl₃) δ 5.88 (dt, 1 H, *J* = 14.4, 6.8 Hz, H-4), 5.50 (dd, 1 H, *J* = 15.2, 6.0 Hz, H-3), 4.61 (d, 1 H, *J* = 6.0 Hz, H-2), 3.80 (s, 3 H, -OMe), 2.80 (s, 1 H, -OH), 2.06 (q, 2 H, *J* = 7.2 Hz, H-5), 1.39 (m, 2 H, H-6), 1.35 – 1.26 (m, 18 H, H-7 to H-15), 0.88 (t, 3 H, *J* = 6.8 Hz, H-16); ¹³C NMR (101 MHz, CDCl₃) δ 174.44 (C-1), 135.27 (C-4), 125.95 (C-3), 71.58 (C-2), 52.92 (-OMe), 32.29, 32.06, 29.82, 29.80, 29.79, 29.75, 29.60, 29.50, 29.29, 28.96, 22.83 (CH₂ x 11), 14.27 (C-16); IR (neat): 3458, 2922, 2853, 1713, 1466 cm⁻¹.

Methyl (R,E)-2-hydroxyhexadec-3-enoate (1.16 g, 4.08 mmol, 1 eq) was dissolved in EtOAc (40 mL) under an argon atmosphere. The solution was purged with argon followed by addition of palladium on carbon (10% loading, 22 mg, 0.2 mmol, 0.05 eq). The mixture was then stirred for 30 minutes under a flow of hydrogen gas and was then left for 60 hours under a hydrogen atmosphere. The mixture was filtered over a pad of Celite and concentration *in vacuo* giving the crude product **16a** as a solid (1.07 g, 3.72 mmol, 91%). *R*_f = 0.2 (10% Et₂O, 10% DCM in pentane); ¹H NMR (400 MHz, CDCl₃) δ 4.19 (dd, 1 H, *J* = 7.2, 4.4 Hz, H-2), 3.79 (s, 3 H, -OMe), 2.69 (bs, 1 H, -OH), 1.78 (m, 1 H, H-3_a), 1.63 (m, 1 H, H-3_b), 1.54 – 1.25 (m, 24 H, H-4 to H-15), 0.88 (t, 3 H, *J* = 6.8 Hz, H-16); ¹³C NMR (101 MHz, CDCl₃) δ 176.02 (C-1), 70.62 (C-2), 52.63 (-OMe), 34.57 (C-3), 32.08, 29.84, 29.83, 29.81 (x2), 29.78, 29.70, 29.61, 29.51, 29.45, 24.88, 22.85 (CH₂ x 12), 14.28 (C-16); IR (neat): 3462, 2920, 2853, 1736, 1460, 1215 cm⁻¹. HRMS (MALDI-TOF) calculated [C₁₇H₃₄O₃ + Ag]⁺: 393.1559, found 393.1572.

α-Hydroxy fatty acid (16b). Methyl ester **16a** (926 mg, 3.23 mmol, 1 eq) was dissolved in THF/MeOH/H₂O (2:2:1, 30 mL) at room temperature. Lithium hydroxide monohydrate (420 mg, 10.0 mmol, 3.1 eq) was added and the reaction mixture was stirred for 20 hours at room temperature.

The reaction mixture was quenched by HCl (1M, 15 mL). The aqueous mixture was extracted with EtOAc (3x 75 mL). The combined organic layers were washed with H₂O (100 mL) and brine, dried over MgSO₄, filtrated and concentrated *in vacuo*. The crude α-hydroxy fatty acid **16b** was collected as a solid (873 mg, 3.20 mmol, 99%) and was used for the next step without further purification. *R*_f = 0.1 (30% EtOAc in pentane); ¹H NMR (400 MHz, CDCl₃) δ 4.28 (dd, 1 H, *J* = 7.6, 4.4 Hz, H-2), 1.86 (m, 1 H, H-3_a), 1.71 (m, 1 H, H-3_b), 1.46 (m, 2 H, H-4), 1.38 – 1.26 (m, 22 H, H-5 to H-15), 0.88 (t, 3 H, *J* = 6.8 Hz, H-16); ¹³C NMR (101 MHz, CDCl₃) δ 179.41 (C-1), 70.38 (C-2), 34.36 (C-3), 32.08, 29.84 (x2), 29.81 (x2), 29.79, 29.71, 29.60, 29.52, 29.41 (CH₂ x 10), 24.89 (C-4), 22.85 (CH₂), 14.29 (C-16); IR (neat): 3445, 2920, 2851, 1732, 1466 cm⁻¹.

Spectroscopic data was identical to literature.^[23]

(R)-2-Acetoxyhexadecanoic acid (16c). The crude α -hydroxy fatty acid **16b** (808 mg, 2.97 mmol, 1 eq) was dissolved in anhydrous DCM (30 mL) under an argon atmosphere at room temperature. Acetic anhydride (4.49 mL, 47.45 mmol, 16 eq) and pyridine (7.45 mL, 92.15 mmol, 31 eq) were added and the reaction mixture was stirred for 20 hours. The reaction was quenched by adding NaHCO₃ (sat. aq., 50 mL). The aqueous layer was extracted with CHCl₃ (2x 50 mL). The combined organic layers were washed with KHSO₄ (0.5 M, 75 mL), dried over MgSO₄, filtrated and concentrated *in vacuo*. The acetylated product **16c** was collected as a yellow solid (905 mg, 2.88 mmol, 97%), which was used for the next step without further purification. R_f = 0.2 (30% EtOAc in pentane); $[\alpha]_D^{25}$ = + 9.0 (c = 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 10.61 (bs, 1 H, -COOH), 5.00 (t, 1 H, J = 6.4 Hz, H-2), 2.14 (s, 3 H, CH₃-Acetyl), 1.86 (m, 2 H, H-3), 1.42 (m, 2 H, H-4), 1.37 – 1.26 (m, 22 H, H-5 to H-15), 0.88 (t, 3 H, J = 6.8 Hz, H-16); ¹³C NMR (101 MHz, CDCl₃) δ 175.96 (C-1), 170.79 (C=O_{Acetyl}), 72.00 (C-2), 32.08 (CH₂), 31.09 (C-3), 29.83 (x2), 29.80 (x2), 29.76, 29.68, 29.51, 29.49, 29.26 (CH₂ x 9), 25.26 (C-4), 22.85 (CH₂), 20.73 (CH₃-acetyl), 14.28 (C-16); IR (neat): 2955, 2916, 2849, 1742, 1722, 1228 cm⁻¹. HRMS (MALDI-TOF) calculated [C₁₈H₃₄O₄ + Ag]⁺: 421.1508, found 421.1516. Spectroscopic data was identical to literature.^[23]

(R)-1-Oxo-1-(((2S,3R,6R,E)-1,3,6-trihydroxyoctadec-4-en-2-yl)amino)hexadecan-2-yl acetate (17). 6-Hydroxy sphingosine (**1** (11 mg, 0.035 mmol, 1 eq) and carboxylic acid **16c** (14 mg, 0.045 mmol, 1.3 eq) were dissolved in anhydrous EtOH (1.5 mL) under an argon atmosphere. 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (**17** mg, 0.069 mmol, 2 eq) was added and the reaction mixture was stirred for 20 hours at 50°C. The reaction mixture was concentrated *in vacuo* and purified with silica gel chromatography (0-3% MeOH in CHCl₃). Acetylated ceramide **17** was collected as a waxy white solid (14 mg, 0.023 mmol, 66%). R_f = 0.3 (5% MeOH in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 6.76 (d, 1 H, J = 8.0 Hz, -NH), 5.84 (dd, 1 H, J = 15.6, 6.4 Hz, H-5), 5.73 (dd, 1 H, J = 15.6, 5.6 Hz, H-4), 4.99 (t, 1 H, J = 6.4 Hz, H-2'), 4.37 (t, 1 H, J = 4.8 Hz, H-3), 4.13 (q, 1 H, J = 6.4 Hz, H-6), 3.97 (dd, 1 H, J = 11.2, 3.2 Hz, H-1_a), 3.89 (m, 1 H, H-2), 3.69 (dd, 1 H, J = 11.2, 3.6 Hz, H-1_b), 3.24 (bs, 1 H, -OH), 2.86 (bs, 1 H, -OH), 2.16 (s, 3 H, CH₃-Acetyl), 1.83 (m, 2 H, H-3'), 1.52 (m, 2 H, H-7), 1.37 – 1.26 (m, 44 H, H-8 to H-17 and H-4' to H-15'), 0.88 (t, 6 H, J = 6.8 Hz, H-18 and H-16'); ¹³C NMR (101 MHz, CDCl₃) δ 170.85, 170.75 (C-1' and C=O_{Acetyl}), 136.27 (C-5), 129.50 (C-4), 74.76 (C-2'), 73.55 (C-3), 72.21 (C-6), 61.94 (C-1), 54.20 (C-2), 37.34 (C-7), 32.08 (CH₂), 31.97 (C-3'), 29.85 (x4), 29.83 (x3), 29.81 (x3), 29.73 (x2), 29.59, 29.51 (x2), 29.41, 25.63, 25.16, 22.84 (CH₂ x 19), 21.10 (CH₃-Acetyl), 14.27 (C-18 and C-16'); HRMS calculated [C₃₆H₆₉NO₆ + Na]⁺: 634.5017, found 634.5011.

Ceramide (2). Acetylated ceramide **17** (11 mg, 0.018 mmol, 1 eq) was dissolved in DCM/MeOH (4:1, 0.2 mL) under an argon atmosphere. Potassium carbonate (catalytic amount) was added and the reaction mixture was stirred for 2 hours at room temperature. The reaction mixture was acidified by adding Amberlyst 1, followed by filtration and concentration *in vacuo*. The crude product was purified with silica gel chromatography (0- 5% MeOH in CHCl₃) giving Ceramide **2** as a white solid (9 mg, 0.016 mmol, 88%). R_f = 0.5 (10% MeOH in CHCl₃); ¹H NMR (850 MHz, CDCl₃/MeOD-*d*₄, 9:1) δ 7.42 (d, 1 H, J = 8.5 Hz, -NH), 5.77 (dd, 1 H, J = 15.3, 6.0 Hz, H-5), 5.66 (dd, 1 H, J = 15.3, 6.0 Hz, H-4), 4.23 (at, 1 H, J = 5.1 Hz, H-3), 4.07 (m, 1 H, H- 6), 4.03 (dd, 1 H, J = 8.5, 3.4 Hz, H-2'), 3.85 (m, 1 H, H-2), 3.81 (dd, 1 H, J = 12.0, 4.3 Hz, H-1_a), 3.69 (dd, 1 H, J = 11.9, 2.6 Hz, H-1_b), 1.58 – 1.44 (m, 4 H, H-7 and H-3'), 1.41 – 1.26 (m, 44 H, H-8 to H-17 and H-4' to H-15'), 0.88 (t, 6 H, J = 6.8 Hz, H-18 and H-16'); ¹³C NMR (214 MHz, CDCl₃/MeOD-*d*₄, 9:1) δ 176.21 (C-1'), 135.79 (C-5), 129.16 (C-4), 72.59 (C-3), 72.18 (C-2'), 71.79 (C-6), 61.45 (C-1), 54.59 (C- 2), 37.12 (C-7), 34.37 (C-3'), 31.96, 29.75, 29.73 (x2), 29.72 (x2), 29.71 (x2), 29.70 (x2), 29.68 (x2), 29.66, 29.63, 29.48, 29.40 (x2), 25.60, 22.72 (CH₂ x 19), 14.11 (C-18 and C-16'); IR (neat): 3377, 3264, 2953, 2916, 2849, 1738, 1715, 1651, 1620, 1470, 1074, 1043 cm⁻¹; HRMS calculated for [C₃₄H₆₈NO₅ + H]⁺: 570.5092, found 570.5087.

5.5 References and notes

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Synthesis of Ceramide-Mimetic Aziridines as Potential Mechanism-Based Enzyme Inhibitors

6.1 Introduction

Glucosylceramide (**1**) is the substrate of at least three hydrolases.^[1] In healthy tissues, glucosylceramide is predominantly processed by lysosomal acid glucosylceramidase (GBA, Figure 6.1) to form glucose (**2**) and ceramide (**3**). Gaucher disease is characterized by genetic impairment of GBA, resulting in glucosylceramide accumulation. Within the lysosomes, elevated glucosylceramide levels can be taken on by acid ceramidase (ACase), which in healthy individuals is responsible for hydrolysis of the amide bond in ceramide to

produce sphingosine (**4**) and a fatty acid (**5**).^[2] In case of increased glucosylceramide levels, acid ceramidase is found to be capable of producing the corresponding glucosylsphingosine (**6**), which is normally not (or only in low quantities) observed, and thus serves as a marker for GBA deficiency. Glucosylceramide (**1**) may also escape from lysosomes to the cytosol, where it can be processed by neutral glucosylceramidase (GBA2).^[3] Also GBA2 produces glucose (**2**) and ceramide (**3**), but does so (in comparison with GBA) in a different subcellular environment: the cytoplasm.

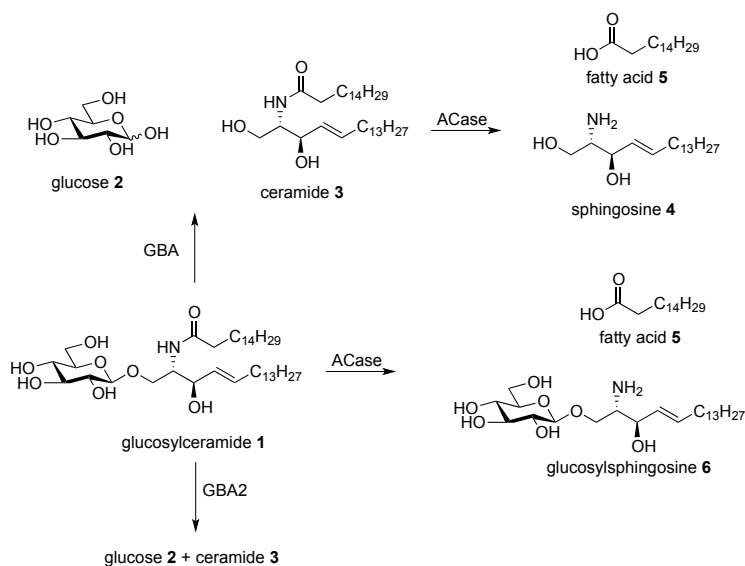


Figure 6.1 Partial overview of metabolism of glucosylceramide **1**. ACCase: acid ceramidase; GBA: glucosylceramidase; GBA2: neutral glucosylceramidase.

In recent years, activity-based probes (ABPs) for each of the three glucosylceramide-processing enzymes, GBA, GBA2^[4,5] and acid ceramidase,^[6] have been developed. In general, the design of an ABP starts with the identification of a covalent, irreversible inhibitor of the enzyme, or enzyme family, at hand. Cyclophellitol (**7**)^[7] is a naturally occurring β -glucopyranose analogue that, upon binding to the enzyme active site, reacts with retaining β -glucosidases to form a covalent, irreversible enzyme-inhibitor adduct (Figure 6.2). Both GBA and GBA2 are retaining β -glucosidases and indeed effective ABPs for both enzymes have been developed based on the cyclophellitol scaffold. Substitution of the primary alcohol in **7** with an azide gave azido-cyclophellitol (**8**)^[8,9] that served as a starting point for the construction (through copper(I)-catalyzed azide-alkyne [2+3] cycloaddition ‘click’ conjugation) of ABPs specific for GBA in the presence of GBA2 and other retaining β -glucosidases. Substitution of the epoxide oxygen for nitrogen and alkylation of the resulting aziridine yielded cyclophellitol aziridine (**9**),^[10] also featuring an azide for click conjugation, yielding in-class, broad spectrum retaining β -glucosidase ABPs

targeting amongst others both GBA and GBA2. An acid ceramidase-recognizing ABP^[6] has been developed based on the covalent and irreversible inhibitor, carmofur (**10**).^[11] Again, installment of an azide (as in **11**) allowed for click ligation of a reporter fluorophore to yield a selective acid ceramidase probe.

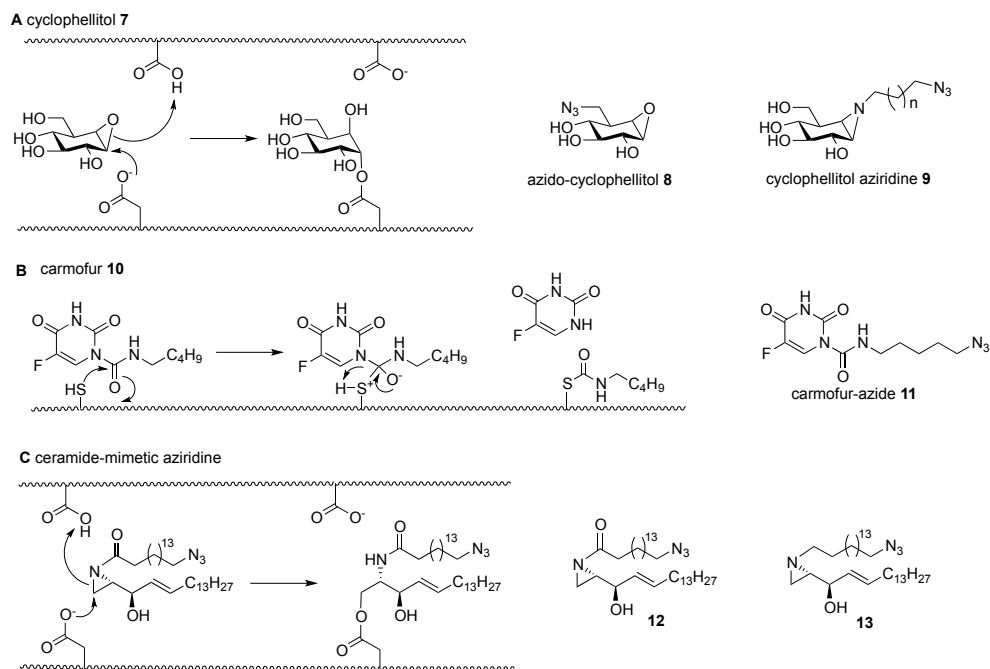


Figure 6.2 A) Mechanism of inhibition of GBA by cyclophellitol **7** and ABP-cyclophellitols **8** and **9**. B) Mechanism of inhibition of acid ceramidase by carmofur **10** and carmofur-azide **11**. C) Possible mechanism of inhibition of GBA/GBA2 and synthetic ceramide aziridine targets **12** and **13** described in this chapter.

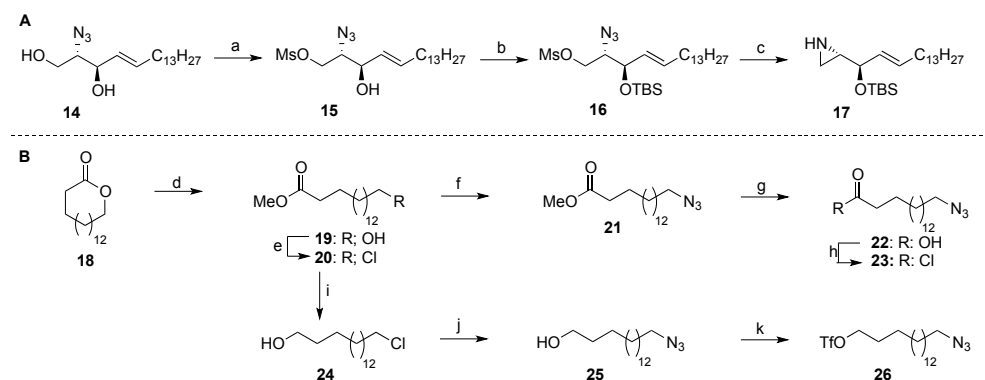
The mode of action of the cyclophellitol-based probes **7**^[8,9] and **8** and their derivatives is based on several features. Cyclophellitols are configurational analogues of β -glucopyranose that adopt a 4H_3 conformation within the enzyme active site.^[12] Once bound, a good leaving group (epoxide-oxygen or aziridine-nitrogen) is positioned optimally for nucleophilic attack by the active-site nucleophilic residue and the electrophilic nature of the epoxide/aziridine is likely enhanced by protonation by the active site acid-base catalyst. Once reacted, an ester bond is formed which is more stable than the acylacetal linkage that emerges during GBA/GBA2-mediated glucosylceramide processing. Looking at this mechanism, one could argue that cyclophellitol emulates only half of the GBA/GBA2 substrate, namely the glucopyranose portion of glucosylceramide (**1**). This in turn invites the question whether an electrophile featuring characteristics of ceramide, being the other half of substrate **1**, would be effective GBA/GBA2 ABPs. With

this idea in mind, ceramide-derived aziridines **12** and **13** (Figure 6.2C), both featuring an azide for bioconjugation or two-step activity-based protein profiling (ABPP) were designed. The synthesis of aziridines **12** and **13** is described in this chapter.

6.2 Results and discussion

The construction of aziridine-ceramides **12** and **13** started from the easily accessible azidosphingosine **14**.^[13] In the first step, selective mesylation of the primary hydroxyl group in **15** was accomplished by reacting methanesulfonyl chloride and 2,4,6-collidine to *in situ* form a mesylcollidinium species,^[14] which due to its steric bulk reacts exclusively with the primary hydroxyl, yielding compound **15**. The allylic hydroxyl in **15** was masked as the TBS-ether (TBSOTf, 2,4,6-collidine).^[15,16] Treatment of the resulting **16** with PPh₃ and water allowed Staudinger reduction of the azide.^[17] The *in situ* formed free amine displaced the primary mesyl group in an intramolecular S_N2 nucleophilic substitution to give partially protected aziridine **17** ready for either N-alkylation or N-acylation towards the two target compounds **12** and **13**.

Scheme 6.1 Synthesis of aziridine **16** and azides **22** and **25**.

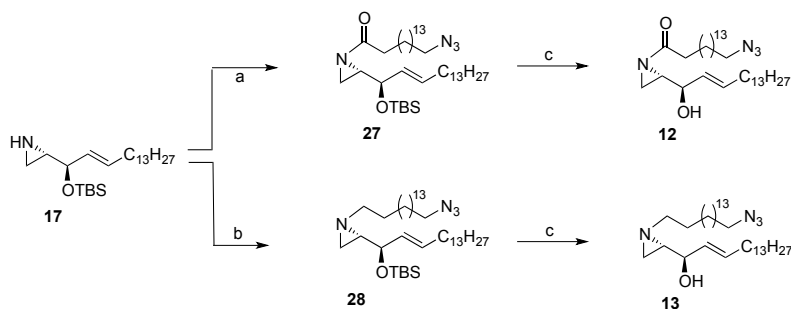


Reagents and conditions: (a) MsCl, 2,4,6-collidine, DCM, 0 °C to 4 °C, 20 h; (b) TBSOTf, 2,4,6-collidine, DCM, 0 °C to 4 °C, 20 h, 65% (over two steps); (c) PPh₃, DIPEA, THF/H₂O (10:1), r.t., 4 h, 59%; (d) NaOMe, MeOH, 60 °C, 3 h, 91%; (e) NCS, PPh₃, THF, r.t., 20 h, 61%; (f) NaN₃, NaI, DMF, 55 °C, 20 h, 94%; (g) LiOH·H₂O, THF/MeOH/H₂O (2:2:1), r.t., 20 h, 61%; (h) oxalyl chloride, DMF, DCM, 0 °C to r.t., 2 h, quant.; (i) LiAlH₄ (1 M in THF), THF, 0 °C, 2 h, 86%; (j) NaN₃, NaI, DMF, 55 °C, 20 h, 93%; (k) Tf₂O, pyridine, DCM, 0 °C, 1 h, quant.

The required 16-azido palmitoyl chloride **23** and 16-azido hexadecanoyl triflate **26** for the construction of these target compounds from aziridine **17** were prepared as follows (Scheme 6.1.B). Trans-esterification of cyclohexadecanolide **18** (NaOMe, methanol) provided methyl ester **19** in 91% yield.^[18] The primary hydroxyl group in **19** was transformed into chloride **20** using Appel conditions (*N*-chlorosuccinimide, triphenylphosphine), after which the chloride was displaced by azide (NaN₃, catalytic NaI)

yielding azide **21** in 55% yield over the three steps based on **18**. Next, the methyl ester in **21** was saponified (LiOH in wet THF/MeOH), giving 16-azido-palmitic acid **22**.^[19] Reaction of **22** with oxalyl chloride gave 16-azido-palmitoyl chloride **23**, which was used for aziridine-*N*-acylation without further purification. In order to enable *N*-alkylation with an azide-functionalized C16-alkane, the methyl ester in **20** was treated with excess LiAlH₄ to yield alcohol **24**. Next, the chloride in **24** was substituted for an azide (NaN₃, catalytic NaI), giving 16-azido hexadecan-1-ol **25** in 94% yield. The primary hydroxyl in **25** was reacted with triflic anhydride and pyridine yielding triflate **26**, which was directly used for *N*-alkylation of aziridine **17**.

Scheme 6.2 Synthesis of aziridine-ceramides **12** and **13**.



Reagents and conditions: (a) **23**, Et₃N, DCM, -10 °C to r.t., 3 h, 61%; (b) **26**, DIPEA, DCM, -20 °C, 3 h; ii) MeOH, 62%; (c) TBAF, THF, r.t., 1 h, 62% **12**, 55% **13**.

With aziridine-sphingosine **17**, acyl azide **23** and alkyl azide **26** in hand, the construction of target compounds **12** and **13** was undertaken. Treatment of aziridine **17** with crude 16-azidopalmitoyl chloride **23** and triethylamine gave compound **27**.^[17] The TBS protecting group in **27** was removed using TBAF in dry THF to afford *N*-acyl-aziridine **12** in 61% yield. *N*-alkylation of aziridine **17** was accomplished by treatment with crude triflate **26** in methanol, providing **28** in 62% yield. Removal of the TBS protecting group in **28** (TBAF, dry THF) gave *N*-alkyl-aziridine **13** in 55% yield.

6.3 Conclusion

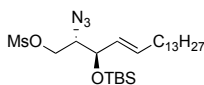
In conclusion, this Chapter describes the synthesis of aziridines **12** and **13** as potential GBA/GBA2 ABPs that are distinguished from the existing ABPs **8** and **9** by emulating the ceramide fragment of the natural substrate (glucosylceramide), rather than the glucose portion. Future research is required to establish whether compounds **12** and **13** are indeed capable of reacting with GBA/GBA2 and to do so in cell extracts or live cells. To this end, compounds **12** and **13** can be conjugated (through click ligation) either prior to or after cell/cell extract incubation, thus in either a direct or two-step ABPP fashion as it has

also been done in the past with azido cyclophellitol **8**.^[9] By performing the ABPP experiments in an unbiased fashion enzymes other than GBA/GBA2 may be identified, enzymes that may be involved in the processing of ceramide derivatives as well and that are characterized by an active site nucleophile that plays a role in enzyme catalysis.

6.4 Experimental section

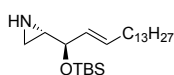
General Remarks. Commercially available reagents and solvents were used as received. DCM and THF were dried and distilled by standard procedures. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out with Silica gel 60 (40–63 µm mesh). IR spectra are reported in cm^{-1} . Optical rotations were measured with an automatic polarimeter (sodium D-line, $\lambda = 589 \text{ nm}$). The enantiomeric purity was determined by HPLC analysis using an OD column (hexane/isopropyl alcohol (98:2), 1 mL/min, UV 254 nm). NMR spectra were recorded on a 400 MHz or 850 MHz spectrometer. Chemical shifts are reported as δ values (ppm), and were referenced to tetramethylsilane ($\delta = 0.00 \text{ ppm}$) directly in CDCl_3 , or using the residual solvent peak (D_2O). High resolution mass spectra were recorded on a LTQ-Orbitrap (Thermo Finnigan) mass spectrometer equipped with an electrospray ion source in positive mode

(2S,3R,E)-2-azido-3-((tert-butyldimethylsilyl)oxy)octadec-4-en-1-yl methanesulfonate (16). Azidosphingosine



14 (1.11 g, 3.39 mmol, 1 eq.) was dissolved in DCM (38 mL) under an atmosphere of argon and 2,4,6-collidine (4.5 mL, 33.9 mmol, 10 eq.) was added. The mixture was left to stir for 15 minutes at 0 °C. MsCl (0.29 mL, 3.73 mmol, 1.1 eq.) was added and the reaction was left to stir for 21 h at 4 °C, after which the reaction was quenched with water. The mixture was diluted with DCM and washed with 1 M HCl (aq.), sat. aq. NaHCO_3 and water. The water layers were extracted with DCM and combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The crude mesylated product **15** was used in the next step without any further purification. The mesylated sphingosine was then dissolved in DCM (35 mL) and 2,4,6-collidine (4.15 mL, 33.9 mmol, 10 eq.) was added. The mixture was left to stir for 15 minutes at 0 °C. TBSOTf (1.46 mL, 6.8 mmol, 2 eq.) was added and the mixture was left to stir over night at 4 °C. The mixture was diluted with DCM and washed with 1 M HCl (aq.), sat. aq. NaHCO_3 and water. The water layers were extracted with DCM and the combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The product was purified by column chromatography (5% acetone in pentane) giving a colorless oil (1.12 g, 2.17 mmol, 65%). $R_f = 0.35$ (5% acetone in pentane); $[\alpha]^{20}_D: -32.8$ ($C = 1.0$, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.71(m, 1 H, H-5), 5.41 (dd, 1 H, $J = 15.8, 7.6 \text{ Hz}$, H-4), 4.31 (dd, 1 H, $J = 10.8, 3.6 \text{ Hz}$, H-3), 4.19 (dd, 1 H, $J = 8.0, 5.3 \text{ Hz}$, H-1), 4.10 (dd, 1 H, $J = 11.0, 8.0 \text{ Hz}$, H-1_b), 3.63 (m, 1 H, H-2), 3.04 (s, 3 H, Me_{MS}), 2.04 (q, 2 H, $J = 6.8 \text{ Hz}$, H-6), 1.36–1.22 (m, 22 H, H-7 to H-17), 0.89–0.81 (m, 12 H, H-18 and Si_{tBu}) 0.07 (s, 3 H, Si_{Me}) 0.03 (s, 3 H, Si_{Me}); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 135.8 (C-5), 128.2 (C-4), 74.2 (C-3), 68.3 (C-1), 65.3 (C-2), 37.7 (CH_3 , Me_{MS}), 32.36, 32.06, 29.83, 29.80, 29.79 x2, 29.59, 29.50, 29.32, 29.02 (11x CH_2 , C-6 to C-17), 25.9 (Si_{tBu}), 22.8 (CH_2 , C-6 to C-17), 14.22 (C-18), -2.85 (Si_{Me}), -4.0 (Si_{Me}); IR (neat): 2924, 2855, 2102, 1360, 1252, 1179, 964, 835, 777 cm^{-1} ; HRMS calculated for $[\text{C}_{25}\text{H}_{51}\text{N}_3\text{O}_4\text{SSi} + \text{H}]^+$: 518.3450, found 518.3464.

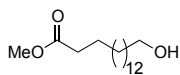
(S)-2-((R,E)-1-((tert-butyldimethylsilyl)oxy)hexadec-2-en-1-yl)aziridine (17). Compound **16** (279 mg, 0.54 mmol,



1 eq.) was dissolved in a mixture of THF/ H_2O (10:1, 4 mL). Triphenylphosphine (230 mg, 0.88 mmol, 1.63 eq.) and DIPEA (190 µL, 1.09 mmol, 2 eq.) were added at room temperature and the reaction mixture was stirred for 4 hours. The mixture was diluted with EtOAc and washed with brine. The water layer was extracted with EtOAc and the combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The product was purified by column chromatography (silica gel, 4–8% acetone/pentane) giving a colorless oil (116 mg, 0.3 mmol, 59%). $R_f = 0.5$ (10% acetone/pentane); $[\alpha]^{20}_D: -30.6$ ($C = 0.66$, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.65 (m, 1 H, H-5), 5.42 (dd, 1 H, $J =$

15.6, 7.0 Hz, H-4), 4.06 (dd, 1 H, $J = 7.2, 4.0$ Hz, H-3), 2.03 (q, 2 H, $J = 7.0$ Hz, H-6), 1.07 (m, 1 H, H-2), 1.62 (d, 1 H, $J = 3.6$, H-1a), 1.52 (d, 1 H, $J = 5.5$ Hz, H-1b), 1.43-1.15 (m, 22 H, H-7 to H-17), 0.87-0.84 (m, 12 H, H-18 and $\text{Si}t\text{Bu}$), 0.04 (s, 3 H, $\text{Si}Me$), 0.02 (s, 3 H, $\text{Si}Me$); ^{13}C NMR (101 MHz, CDCl_3) δ 132.7 (C-5), 131.0 (C-4), 72.2 (C-3), 35.0 (C-2), 32.3, 32.1, 29.85, 29.83 x2, 29.81 x2, 29.77, 29.63, 29.51, 29.32, 29.27, (12x CH_2 , C-1 and C-6 to C-17), 26.0 ($\text{Si}t\text{Bu}$), 22.8, (CH_2 , C-6 to C-17), 14.3 (C-18), -3.9 ($\text{Si}Me$), -4.7 ($\text{Si}Me$); IR (neat): 2924, 2853, 1462, 1252, 1060, 906, 835, 731 cm^{-1} . HRMS calculated for $[\text{C}_{24}\text{H}_{49}\text{NOSi} + \text{H}]^+$: 396.3663, found 396.3653.

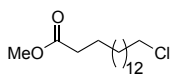
methyl 16-hydroxyhexadecanoate (19). Cyclohexadecanolide **18** (2.6 g, 10.2 mmol, 1 eq.) was dissolved in dry



MeOH (60 mL) under an atmosphere of argon. To the solution was added NaOMe (30% in methanol, 9.3 mL, 50 mmol, 5 eq.) was added. The reaction mixture was refluxed for 3 hours at 65 °C after which it was cooled to room temperature. The reaction was

quenched with 1 M HCl (aq.) to a pH of 11. The mixture was diluted with EtOAc, washed with sat. aq. NaHCO_3 . The water layer was extracted with EtOAc and combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The product was purified by column chromatography (Silica gel, 1:2 EtOAc/pentane) giving a white solid (2.62 g, 9.11 mmol, 91 %). $R_f = 0.75$ (1:2 EtOAc/pentane). ^1H NMR (400 MHz, CDCl_3) δ 3.66 (s, 3 H, OMe), 3.64 (t, 2 H, $J = 7.2$ Hz, H-16), 2.30 (t, 2 H, $J = 7.8$ Hz, H-2) 1.65-1.51 (m, 4 H, H-3 and H-15), 1.28-1.25 (m, 22 H, H-4 to H-14). ^{13}C NMR (101 MHz, CDCl_3) δ 174.5 (C-1), 63.2 (C-16), 51.6 (C-OMe), 34.3 (C-2), 33.0 (C-15), 29.77 x2, 29.76, 29.74, 29.72, 29.58, 29.39, 29.29, 25.88, 25.10 (12x CH_2 , C-3 to C-14); IR (neat): 2918, 2849, 1738, 1161 cm^{-1} .

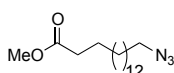
methyl 16-chlorohexadecanoate (20). Compound **19** (2.62 g, 9.1 mmol, 1 eq.) was dissolved in dry THF (60 mL)



under an atmosphere of argon. To the solution was added PPh_3 (2.64 g, 10.02 mmol, 1.1 eq.) and NCS (1.39 g, 10.02 mmol, 1.1 eq.). The reaction stirred over night at room temperature. The mixture was diluted with EtOAc and washed with water and brine. The

water layers were extracted with EtOAc and combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The crude product was purified by column chromatography in (Silica gel, 0% to 4% EtOAc/pentane) giving a colorless oil (1.69 g, 5.53 mmol, 61%). $R_f = 0.8$ (2 % EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 3.66 (s, 3 H, -OMe), 3.53 (t, 2 H, $J = 7.0$ Hz, H-16), 2.30 (t, 2 H, $J = 7.6$ Hz, H-2) 1.76 (p, 2 H, $J = 7.2$ Hz, H-15), 1.62 (m, 2 H, H-3), 1.42 (m, 2 H, H-14), 1.28-1.26 (m, 18 H, H-4 to H-13); ^{13}C NMR (101 MHz, CDCl_3) δ 174.4 (C-1), 51.5 (-OMe), 45.2 (C-16), 34.2 (C-2), 32.8, 29.7 x3, 29.68, 29.65, 29.57, 29.55, 29.36, 29.26, 29.00, 27.0, 25.1 (13x CH_2 , C-3 to C15); IR (Neat): 2922, 2853, 1740, 1435, 1169, 721 cm^{-1} .

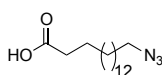
methyl 16-azidohexadecanoate (21). Chloride **20** (856 mg, 2.8 mmol, 1 eq.) was dissolved in DMF (13 mL) and



NaN_3 (580 mg, 8.9 mmol, 3 eq.) and a catalytic amount of NaI were added. The reaction was stirred over night at 55 °C. The mixture was diluted with ether, washed with water and brine and extracted. The water layers were extracted with Ether and the combined

organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, 1-2% acetone in pentane) giving a white solid (791 mg, 2.54 mmol, 94%). $R_f = 0.8$ (2% acetone/pentane); ^1H NMR (400 MHz, CDCl_3) δ : 3.65 (s, 3 H, OMe), 3.24 (t, 2 H, $J = 7.0$ Hz, H-16), 2.29 (t, 2 H, $J = 7.4$ Hz, H-2), 1.63-1.55 (m, 4 H, H-3 and H-15), 1.38-1.26 (m, 22H, H-4 to H-14); ^{13}C NMR (101 MHz, CDCl_3) δ : 173.9 (C-1), 51.3 (C-16), 51.2 (CH_3 , C-OMe), 33.9 (C-2), 29.56 x3, 29.52, 29.48, 29.43, 29.39, 29.02, 29.09, 29.08, 29.87, 26.66, 24.87 (13x CH_2 , C-3 to C-15); IR (neat): 2922, 2853, 2093, 1740, 1252, 1169 cm^{-1} . HRMS calculated for $[\text{C}_{16}\text{H}_{31}\text{N}_3\text{O}_2 + \text{H}]^+$: 298.2496, found 298.2509.

16-azidohexadecanoic acid (22). Compound **21** (791 mg, 2.54 mmol, 1 eq.) was dissolved in a mixture of



THF/MeOH/ H_2O (2:2:1, 25 mL) and $\text{LiOH} \cdot \text{H}_2\text{O}$ (337 mg, 7.88 mmol, 3.1 eq.) was added. The reaction stirred over night at room temperature, after which it was acidified with 1 M HCl (aq) to a pH of 1-2. The mixture was diluted with EtOAc and extracted with water and brine.

The water layers were extracted with EtOAc and combined organic layers were dried (MgSO_4), filtered and

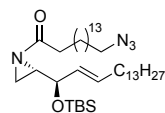
concentrated *in vacuo* giving a white solid (446 mg, 1.5 mmol, 59%), which was used without any further purification. $R_f = 0.2$ (1:1:8 ether/DCM/pentane); ^1H NMR (400 MHz, CDCl_3) δ 3.26 (t, 2 H, $J = 7.0$ Hz, H-16), 2.35 (t, 2 H, $J = 7.4$ Hz, H-2), 1.67-1.56 (m, 4 H, H-3 and H-15), 1.36-1.22 (m, 22 H, H-4 to H-14). ^{13}C NMR (101 MHz, CDCl_3) δ 180.2 (C-1), 51.6 (C-16), 34.0 (C-2), 29.7, 29.6, 29.5, 29.3, 29.2, 29.0, 26.7, 24.9 (13x CH_2 , C-3 to C-15); IR (neat): 2913, 2847, 2112, 1699, 1290 cm^{-1} . HRMS calculated for $[\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_2 + \text{H}]^+$: 284.2340, found 284.2335.

16-azidohexadecanoyl chloride (23). Compound **22** (151 mg, 0.5 mmol, 1 eq.) was dissolved in dry DCM (1.6 mL) and the mixture was cooled to 0 °C. Oxalyl chloride (85 μL , 1 mmol, 2 eq.) was added, followed by 1 drop of DMF, which released gas. The reaction was allowed to warm to room temperature. After no more gas was released, another drop of DMF was added. This process was repeated until no more gas was formed as DMF was added. The solvent was removed *in vacuo* and not purified any further, as the crude was immediately used in the production of compound **27**. Quantitative yield was assumed. $R_f = 0.85$ (10% acetone/pentane); ^1H NMR (400 MHz, CDCl_3) δ 3.25 (t, 2 H, $J = 7.0$ Hz, H-16), 2.88 (t, 2 H, $J = 7.2$ Hz, H-2), 1.74-1.66 (m, 2 H, H-3), 1.63-1.56 (m, 2 H, H-15), 1.32-1.26 (m, 22 H, H-4 to H-14); ^{13}C NMR (101 MHz, CDCl_3) δ 173.8 (C-1), 51.5 (C-16), 47.2 (C-2), 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 28.9, 28.5, 26.8, 25.10 (13C, C-3 to C-15). IR (neat): 2914, 2849, 2097, 1800, 1701 cm^{-1} .

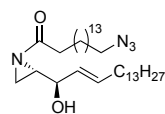
16-Chloro-hexadecan-1-ol (24). Methyl ester **20** (0.6 g, 2 mmol, 1 eq) was dissolved in dry diethyl ether (10 mL) under protected atmosphere and cooled to 0 °C. LiAlH_4 (1 M in THF, 2.5 mL, 2.5 mmol, 1.25 eq) was added drop wise and the reaction mixture was stirred for 2 hours at 0 °C. The reaction was then quenched with 1 M HCl, followed by filtration to remove inorganic salts. The diethyl ether was separated from the water layer, dried (MgSO_4), filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (10% EtOAc in Pentane) giving a white solid (0.47 g, 1.72 mmol, 86%). $R_f = 0.60$ (20% EtOAc in Pentane); ^1H NMR (400 MHz, CDCl_3) δ 3.64 (t, 2 H, $J = 6.8$ Hz, H-1), 3.53 (t, 2 H, $J = 6.8$ Hz, H-16), 1.77 (p, 2 H, $J = 6.8$ Hz, H-15), 1.57 (p, 2 H, $J = 6.8$ Hz, H-2), 1.42 (m, 2 H, H-14), 1.35-1.22 (m, 14 H, H-3 to H-13); ^{13}C NMR (101 MHz, CDCl_3) δ 63.2 (C-1), 45.4 (C-16), 32.94 (C-2), 32.79 (C-15), 29.78, 29.75, 29.73, 29.68, 29.60, 29.57, 29.03, 27.03, 25.87 (12 x CH_2 C-3 to C-14). IR (neat) 3279, 2922, 2853, 1464, 1055, 721 cm^{-1} .

16-Azido-hexadecan-1-ol (25). 16-chloro-pentanol **24** (0.4 g, 1.45 mmol, 1 eq) was dissolved in dry DMF (10 mL) followed by addition of NaN_3 (0.19 g, 2.9 mmol, 2 eq) and catalytic amount of NaI. The mixture was heated to 60 °C and left stirring over night. The reaction mixture was diluted with diethyl ether and washed with water and brine. The water layers were extracted with diethyl ether and the combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The product was purified with silica column chromatography (10% EtOAc in Pentane) giving a white solid (0.37 g, 1.31 mmol, 94%). $R_f = 0.60$ (20% EtOAc in Pentane); ^1H NMR (101 MHz, CDCl_3) δ 3.64 (t, 2 H, $J = 6.8$ Hz, H-1), 3.25 (t, 2 H, $J = 7.2$ Hz, H-16), 1.63-1.53 (m, 4 H, H-2 and H-15), 1.40-1.26 (m, 24 H, H-3 to H-14); ^{13}C NMR (101 MHz, CDCl_3) δ 63.2 (C-1), 51.2 (C-16), 33.01 (C-2), 29.55, 29.51, 29.47, 29.41, 29.37, 29.19, 29.08, 29.07, 28.77, 26.64, 24.85 (13x CH_2 C-3 to C-15); IR (neat) 3294, 2922, 2853, 2098, 1464, 1055 cm^{-1} .

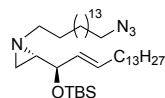
16-Azido-hexadecan-1-O-triflate (26). 16-Azido-hexadecan-1-ol **25** (57 mg, 0.2 mmol, 1.0 eq) was dissolved in dry DCM (2 mL) under protected atmosphere and cooled to 0 °C. Pyridine (19 μL , 0.24 mmol, 1.2 eq) was added followed by addition of Tf_2O (41 μL , 0.24 mmol, 1.2 eq). The mixture was stirred for 1 hour followed by dilution with DCM (10 mL). The reaction was washed with water and brine. The water layers were extracted with DCM and the combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo* giving the crude triflate, which was directly used without any further purification in the next reaction.

1-((S)-2-((R,E)-1-((tert-butyldimethylsilyl)oxy)hexadec-2-en-1-yl)aziridin-1-yl)hexadecan-1-one (27).

sphingosine **17** (101 mg, 0.26 mmol, 1 eq.) was dissolved in DCM (5 mL) and triethylamine (62 μ L, 0.44 mmol, 1.73 eq.) was added. The mixture was cooled to -10°C . 16-azido-palmitoyl chloride **23** (0.5 mmol, 1.9 eq.) was added drop-wise and the mixture stirred and was allowed to warm to room temperature over 3 h. The solvent was removed *in vacuo* to give a crude product. The crude was purified by column chromatography giving a colorless oil. (silica gel, 0% to 2% acetone/pentane). Yield (155 mg, 0.23 mmol, 90%), R_f = 0.65 (5% acetone/pentane); $[\alpha]^{20}_D$: -27.0 ($C=0.2$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 5.81-5.78 (m, 1 H, H-5), 5.58 (dd, 1 H, $J=15.6, 8.0$ Hz, H-4), 4.20-4.18 (m, 2 H, H-3), 3.37 (t, 2 H, $J=7.0$ Hz, H-16*) 2.58-2.54 (m, 1 H, H-2), 2.45-2.41 (m, 1 H, H-1_a), 2.20 (d, 1 H, $J=3.2$ Hz, H-1_b), 2.17-2.13 (m, 2 H, H-6), 1.76-1.69 (m, 4 H, H-2* and H-15*), 1.48-1.37 (m, 46 H, H-7 to H-17 and H-3* to H-14*), 1.00-0.98 (m, 12 H, H-18 and Si_{tBu}), 0.16 (s, 3 H, Si_{Me}), 0.15 (s, 3 H, Si_{Me}); ^{13}C NMR (101 MHz, CDCl_3) δ 133.5 (C-5), 129.5 (C-4), 73.2 (C-3), 51.6 (C-16*), 41.1 (C-2), 36.8 (C-6), 32.3, 32.9, 29.7, 29.6, 29.4, 29.3, 29.2, 28.9, 27.8 (C-1, C-7 to C-17 and C-2* to C-15*), 26.8 (3x CH_3 , Si_{tBu}), 25.9, 25.3, 22.8 (C-1, C-7 to C-17 and C-2* to C-15*), 14.2 (C-18), -4.0 (2x CH_3 , Si_{Me}); IR (neat): 2922, 2853, 2093, 1703, 1464, 1250, 970, 835 cm^{-1} . HRMS calculated for $[\text{C}_{39}\text{H}_{74}\text{N}_4\text{O}_2\text{Si} + \text{H}]^+$: 675.5974, found 675.5998.

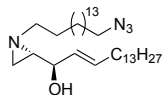
1-((S)-2-((R,E)-1-hydroxyhexadec-2-en-1-yl)aziridin-1-yl)hexadecan-1-one (12).

aziridine-ceramide **27** (0.23 mmol, 1 eq.) was dissolved in THF (1.5 mL) and TBAF (1M in THF, 275 μ L, 0.28 mmol, 1.2 eq.) was added. The reaction stirred for 1 h. at room temperature. The mixture was diluted with EtOAc/ H_2O and washed with brine and extracted. The organic layer was dried over MgSO_4 , filtered and concentrated *in vacuo*. The yielded product was purified by column chromatography giving a colorless oil. (Silica gel, 10% to 20% acetone/pentane) Yield (81 mg, 0.14 mmol, 61%), R_f = 0.5 (10% acetone/pentane), $[\alpha]^{20}_D$: -16.2 ($C=1.0$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 5.84-5.75 (m, 1 H, H-5), 5.44 (dd, 1 H, $J=15.6, 8.0$ Hz, H-4), 5.08 (t, 1 H, $J=10.4$ Hz, H-3), 3.26 (t, 2 H, $J=9.2$ Hz, H-34), 2.32 (t, 2 H, $J=10$ Hz, H-20), 2.24-2.18 (m, 1 H, H-2), 2.05-2.03 (m, 2 H, H-6), 1.78 (d, 1 H, $J=7.6$ Hz, H-1), 1.62-1.55 (m, 5 H, H-1, H-21 and H-33), 1.26 (s, 44 H, H-7 to H-17 and H-22 to H-32), 0.88 (t, 3 H, $J=8.8$ Hz, H-18); ^{13}C NMR (101 MHz, CDCl_3) δ 173.1 (C-19), 136.6 (C-5), 124.9 (C-4), 75.6 (C-3), 51.7 (C-34), 34.7 (C-20), 32.5, 32.2 (25C, C-1, C-21 to C-33), 32.1 (C-2), 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.0, 26.9, 25.2, 23.1, 22.9 (25C, C-1, C-21 to C-33), 14.3 (C-18). IR (Neat): 2914, 2849, 2095, 1726, 1468, 1177, 968 cm^{-1} ; HRMS calculated for $[\text{C}_{34}\text{H}_{64}\text{N}_4\text{O}_2 + \text{H}]^+$: 561.5109, found 561.5138.

Alkylaziridine sphingosine (28).

aziridine **17** (58 mg, 0.15 mmol, 1.0 eq) was dissolved in dry DCM (1.5 mL) under protected atmosphere and cooled -20°C . DIPEA (29 μ L, 0.16 mmol, 1.1 eq) was added followed by addition of triflate **26** (1 M in DCM, 1.6 mL, 0.16 mmol, 1.1 eq) and was left stirring for 3 hours. The reaction mixture was quenched with MeOH (0.1 mL) and washed with water and brine. The water layers were extracted with DCM and combined organic layers were dried (MgSO_4) filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (pentane to 2% acetone in pentane) giving a colorless oil (63 mg, 0.09 mmol 62%). $[\alpha]^{20}_D$: 44 ($C=0.5$, CHCl_3); R_f = 0.72 (5% acetone in pentane); ^1H NMR (400 MHz, CDCl_3) δ 5.61 (m, 1 H, H-5), 5.53 (dd, 1 H, $J=12.4, 6.0$ Hz, H-4), 3.55 (t, 1 H, $J=6.8$ Hz, H-3), 3.25 (t, 2 H, $J=7.2$ Hz, H-16*), 2.43 (m, 1 H, H-1*), 2.01 (q, 1 H, $J=7.2$ Hz, H-6), 1.93 (m, 1 H, H-1_b*), 1.66 (d, 1 H, $J=3.2$ Hz, H-1_a), 1.61 (m, 1 H, H-2*), 1.41-1.25 (m, 48 H, H-1_b, H-2, H-7 to H-17 and H-3* to H-15*), 0.88 (m, 12 H, H-18 and Si_{tBu}), 0.02 (s, 3 H, Si_{Me}), 0.01 (s, 3 H, Si_{Me}); ^{13}C NMR (101 MHz, CDCl_3) δ 134.96 (C-5), 127.79 (C-4), 76.13 (C-3), 61.39 (C-1*), 51.62 (C-16*), 44.80 (C-2), 33.48, 32.33, 32.08, 29.97, 29.70, 29.64, 29.41, 29.36, 29.31, 28.98, 27.57, 26.86, 26.71, 26.86, 26.71 (C-1, C-6 to C-17 and C-2* to C-15*), 22.84 (Si_{tBu}), 18.38 ($\text{C}_{\text{q-Si-tBu}}$), 14.27 (C-18), $-4.29, -4.39$ (2x $\text{C}_{\text{Si-Me}}$); IR (neat); 2922, 2852, 2094, 1463, 1249, 1066, 835, 775 cm^{-1} ; HMRS calcd for $[\text{C}_{40}\text{H}_{80}\text{N}_4\text{OSi} + \text{H}]^+$: 661.6181, found 661.6206.

Alkylaziridine sphingosine (13). The alkylated aziridine **28** (52 mg, 0.08 mmol, 1.0 eq) was dissolved in dry THF (0.8 mL) under an atmosphere of Argon. TBAF (1 M in THF, 0.1 mL, 0.1 mmol, 1.25 eq) was added and the reaction was stirred for 2 hours at room temperature. The reaction was diluted with EtOAc and washed with water and Brine. The water layers were extracted with EtOAc and combined organic layers were dried (MgSO₄), filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (5 % Acetone in Pentane) giving waxy white solid (24 mg, 0.044 mmol, 55%). [α]_D²⁰: 25 (C= 0.25, CHCl₃); R_f = 0.56 (10% Acetone in Pentane); ¹H NMR (400 MHz, CDCl₃): 5.73 (dt, 1 H, *J* = 15.6, 8.0 Hz, H-5), 5.38 (dd, 1 H, *J* = 15.6, 7.6 Hz, H-4), 4.15 (m, 1 H, H-3), 3.25 (t, 2 H, *J* = 6.8 Hz, H-16*), 2.41 (m, 1 H, H-1a*), 2.19 (m, 1 H, H-1b*), 2.03 (m, 2 H, H-6), 1.81 (d, 1 H, *J* = 3.2 Hz, H-1a), 1.62-1.53 (m, 5 H, H-1b, H-2* and H-15*), 1.35-1.25 (m, 45 H, H-2, H-7 to H-17 and H-3* to H-14*), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18); ¹³C NMR (101 MHz, CDCl₃): 134.00 (C-5), 129.39 (C-4), 70.05 (C-3), 60.28 (C-1*), 51.63 (C-16*), 42.71 (C-2), 32.47, 32.07, 29.81, 29.77, 29.69, 29.65, 29.54, 29.51, 29.35, 29.30, 29.24, 28.98, 28.77, 27.47, 26.86, 25.66, 22.84 (C-1, C-6 to C-17 and C-2* to C-15*), 14.27 (C-18); IR (neat) 2921, 2850, 2094, 1467, 1177, 968 cm⁻¹. HRMS calculated for [C₃₄H₆₆N₃O + H]⁺: 547.5317, found 547.5339.



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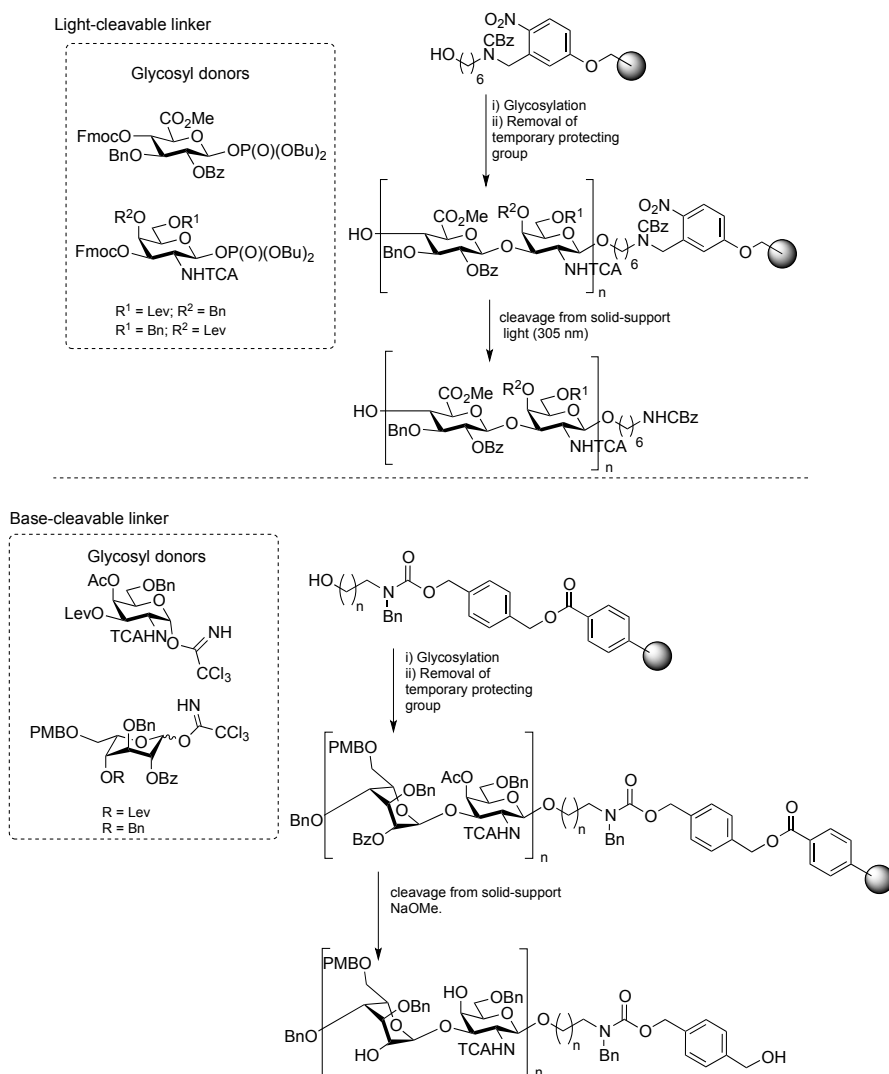
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Chapter 7

Summary and Future Prospects

Sphingolipids constitute a broad class of biomolecules that play key roles in numerous physiological processes in various organisms. The availability of synthetic sphingolipid derivatives, both the natural products themselves (which may be stable-isotope-enriched) and synthetic analogues, is key to unravel sphingolipids biology. The work described in this Thesis focused on the development of synthetic methodology towards modified sphingolipids. Specifically, methodology has been developed for the synthesis of both glycosphingolipids and phosphosphingolipids, both classes of compounds were prepared

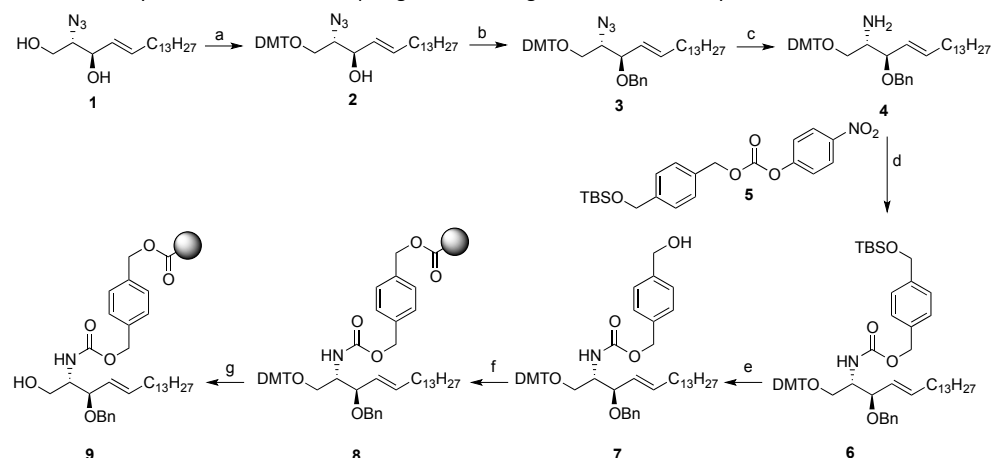
in natural abundance and stable-isotope-enriched forms. As well, methodology has been developed that allowed the synthesis of a rare hydroxylated sphingosine base, as well as unnatural, azide-modified sphingoid bases. Although not discussed in depth in this Thesis, the compounds prepared during this PhD work are highly useful in chemical biology and metabolomics studies on biological systems and processes in which sphingolipid metabolism plays a key role. These include human inherited disorders in which a specific enzyme involved in sphingolipid metabolism is impaired, such as the lysosomal storage disorders, Gaucher disease and Fabry disease. **Chapter 1** introduces sphingolipid biology with a focus on the current status in the literature on chemically prepared, sphingolipid-derived reporter molecules by reviewing the literature of existing sphingolipid analogues including those that are isotopically labeled, equipped with a fluorophore, or outfitted with a bioorthogonal ligation handle. **Chapter 2** describes the synthesis of a panel of carbon-13-labeled (glyco-)sphingolipids based on a cross-metathesis event as the key step. The linear, terminal $^{13}\text{C}_5$ -1-pentadecene for this purpose was assembled from $^{13}\text{C}_2$ -acetic acid and potassium ^{13}C -cyanide using, amongst other transformations, Horner-Wadsworth-Emmons chemistry. After assembling the partially protected sphingosine base (which was prepared both in $^{13}\text{C}_5$ -enriched and natural abundance-carbon form), different donor glycosides (glucosyl and GB3) were condensed with the free primary alcohol to yield, after further chemical manipulations, the corresponding glycosylsphingosines. *N*-acylation of the free amine of these with palmitoyl chloride ($^{13}\text{C}_0$ or $^{13}\text{C}_3$) produced the corresponding glycosylceramides. Although all target compounds could be obtained in good quantity and purity, a caveat of the procedure described in Chapter 2 is the low yield in the glycosylation steps, which can be correlated to the acid-sensitivity of the *N*-Boc protective group chosen. With the aim to improve on the methodology, **Chapter 3** introduces the use of the Fmoc group, instead of the Boc group, as a means to protect the secondary amine in the sphingoid base. This change of *N*-protective group necessitated also changing the nature of the protective group for the secondary alcohol in the sphingoid base, as is outlined in Chapter 3. The net result of the studies described in Chapter 3 is an improvement in yield in the glycosylation step, and $^{13}\text{C}_6$ -glucosylsphingosine, 6-azido-6-deoxy-glucosylsphingosine as well as galactosylsphingosine were readily prepared following the new strategy, in good yield and in excellent purity.

Scheme 7.1 Automated solid-phase oligosaccharide synthesis using light^[2]- or base^[5] sensitive linkers.

An alternative strategy to the solution synthesis of carbohydrates that has received considerable attention in recent years comprises (automated) solid phase oligosaccharide synthesis (see for examples Scheme 7.1).^[1] Key in solid phase carbohydrate synthesis campaigns are full control over stereochemical outcome in the formation of glycosidic linkages, as well as the availability of appropriate linker systems that allow assembly of the oligosaccharides on a solid support, and that can be cleaved at the end of synthesis campaign. With respect to the latter, the Seeberger group has reported two linker systems that may also turn out to be useful in the assembly, on solid support, of

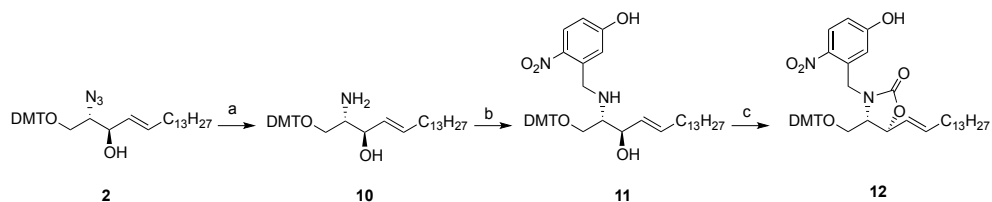
glycosphingolipids: a photo-cleavable^[2,3] linker (sensitive to 305 nm light) and a base-labile linker.^[4,5] Both linker systems are on paper suitable for the construction of glycosphingolipids on solid support, as is outlined in Schemes 7.2 and 7.3. Protective group manipulations on azidosphingosine **1** affords in three steps and good yield partially protected sphingosine **4**, with the amine free for coupling to with the base-sensitive linker (**4** to **5**). After coupling to a solid support (**6** to **8**) and selective unmasking of the primary alcohol (removal of the acid-labile DMT group, **8** to **9**) an immobilized sphingosine derivative was obtained and that is ready for study on its suitability as an acceptor in ensuing glycosylation events.

Scheme 7.2 Synthesis of immobilized sphingosine featuring a base-labile linker system.



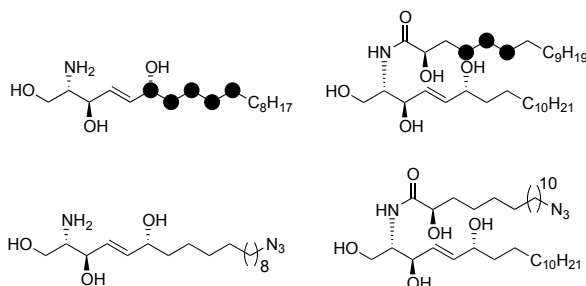
Reagents and conditions: (a) DMTCl, 2,4,6-collidine, DCM, 0 °C, 2 h, 92%; (b) (i) NaH, DMF, 0 °C, 15 min; (ii) BnBr, TBAI, 0 °C to r.t., 20 h, 88%; (c) (i) PMe₃, wet THF, r.t., 20 h; (d) DIPEA, DMF, 0 °C to r.t., 20 h, 75%; (e) TBAF (1 M in THF), THF, r.t., 2 h, 98%; (f) DIC, DMAP, DCM, 20 h; (g) TCA, DCM, 5 min.

In a related strategy amine **10**^[6] (obtained from **2** in one step) can be transformed into carbamate **12**, with the carbamate nitrogen functionalized with a photosensitive 2-nitro-4-hydroxytoluyl moiety (Scheme 7.3). Further elaboration of **12** may yield an immobilized sphingoid acceptor ready for glycosylation, featuring a photosensitive linker for cleavage of the fully assembled glycosphingolipid. Both **9** and **12** will, after a successful solid phase oligosaccharide synthesis scheme, cleavage from the resin and global deprotection, yield glycosylsphingosines. Acylation of the free amine will then yield the corresponding glycosylceramides. Obviously, the feasibility of **9** and **12** as building blocks for solid phase synthesis schemes requires considerable synthetic studies, as the stereospecific introduction of glycosidic linkages is not guaranteed and stereoselective outcome may vary going from one donor-acceptor pair to the next.

Scheme 7.3 Synthesis light-cleavable sphingosine solid-support.

Reagents and conditions: (a) H_2 (g), Lindlar cat., EtOH, r.t., 20 h, 54%; (b) (i) 5-hydroxy-2-nitrobenzaldehyde, MeOH, r.t., 1 h; (ii) NaBH_3CN , r.t., 20 h, 65%; (c) CDI, DCM, r.t., 20 h, 90%

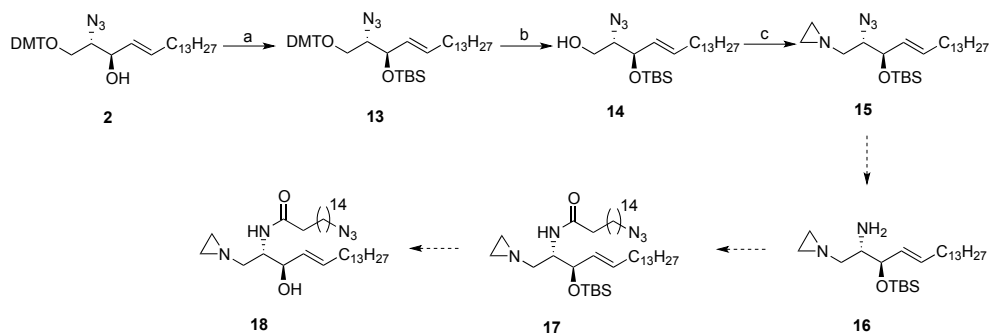
Chapter 4 describes the synthesis of a panel of carbon-13-labeled phosphosphingolipids. Key in these studies, apart from identifying the optimal order of transformations, was the identification of suitable solvent systems to dissolve the sphingosine-1-phosphate derivatives, both for purification and acylation of the sphingosine-amine. **Chapter 5** presents an efficient synthesis of 6-hydroxysphingosine and α -hydroxy palmitic acid in which cross-metathesis features as a key step. The main synthetic challenge proved to be the Grubbs cross-metathesis between two different allylic alcohols, to avoid cross metathesis between two copies of the same alkene. To discriminate between the allylic hydroxyls, the amino-alcohol system of the sphingosine head group (involving the secondary, allylic alcohol) was protected as a cyclic carbamate, while the allylic hydroxyl of the hydroxyalkene was left free. Furthermore, addition of CuI to form a Grubbs 2nd-catalyst CuI complex proved essential to obtain an efficient cross-metathesis between the two alkenes. Extension of the strategy may involve the introduction of ^{13}C isotopes, fluorophores or a ligation handle (Scheme 7.4). Such modified 6-hydroxysphingosines may be useful for visualization of, for instance, 6-hydroxysphingosine trafficking in mammalian cells.

Scheme 7.4 Example of possible modifications of 6-hydroxysphingosines and α -hydroxy fatty acids.

Chapter 6 describes the synthesis of two ceramide-mimetic aziridines which were designed as covalent inhibitors for enzymes involved in glycosphingolipid processing, including the glucosylceramidases, GBA and GBA2. In case successful, the azide in both compounds may be utilized as bioorthogonal ligation handles, thus rendering the

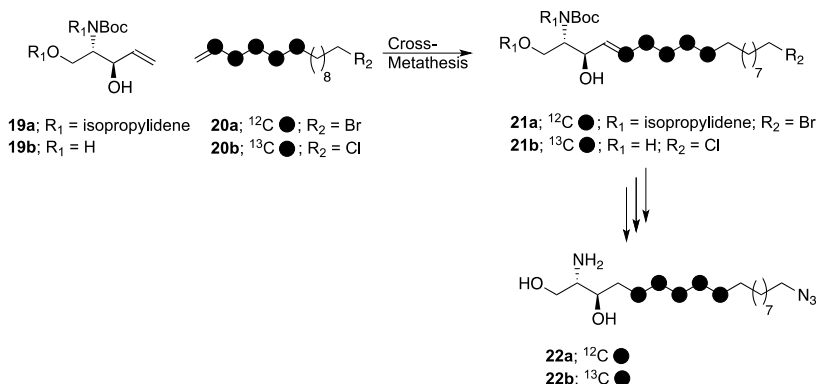
aziridines into potential two-step activity-based probes. Another interesting ceramide-mimetic aziridine is compound **18**, in which the primary hydroxyl has been substituted by an aziridine (Scheme 7.5). The synthesis of **18** started from DMT-protected sphingosine **2**, which was silylated with TBSOTf and 2,4,6-collidine resulting in sphingosine **13**. DMT removal (TFA, addition of dodecanethiol as scavenger)^[9], subsequent triflation (Tf₂O, pyridine) followed by *N*-alkylation with ethyleneimine^[10] yielded aziridine **15**. Azide reduction, *N*-acylation and TBS removal should yield aziridine **18** as a potential alternative sphingosine-derived ABP.

Scheme 7.5 Synthesis towards 1-aziridine ceramide **18**.

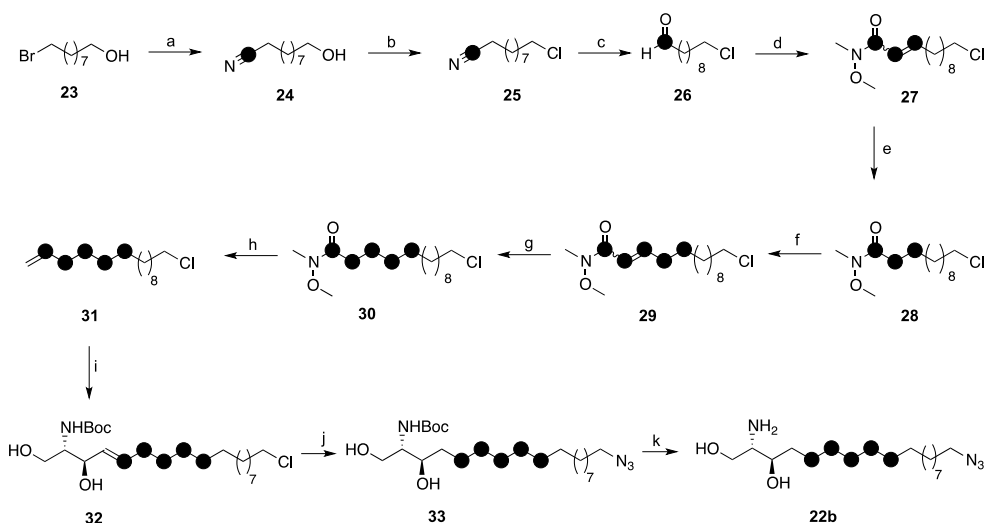


Reagents and conditions: (a) TBSOTf, 2,4,6-collidine, DCM 0 °C, 20 h, 65%; (b) dodecanethiol, TFA, DCM, 0 °C, 80%; (c) (i) Tf₂O, pyridine, DCM, 0 °C, 1 h; (ii) ethyleneimine, DCM, r.t., 20 h, 45%.

APBs have been proven to be excellent tools to study sphingolipid metabolic enzymes, but in order to get insight in sphingolipid pools as a function of these enzymes another chemical biology approach may be more suited. In a recent article by Delgado and co-workers^[11], the sphingolipidome was studied by exposing cells to azide-modified sphinganine **22a** (Scheme 7.6). All sphingolipids are derived from sphinganine and therefore treatment of cells with azido-sphinganine should result in azide tagging of the complete sphingolipidome. The azide can, following lysis of the tissue culture and isolation of the lipid fraction, be addressed as a ligation handle for introduction of, for instance, a fluorophore. Combining the strategy of Delgado and co-workers with the stable-isotope-encoding strategy presented in this Thesis would lead to ¹³C₅-labeled azidosphinganine **22b** that, together with its non-isotopically enriched counterpart **22a** may be used for quantitative, chemical metabolomics studies.^[12] Cross metathesis of either aminodiol **19a** or **19b** with halogenated alkene **20a/b** yielded sphingosine derivatives **21** and **21b**, respectively. Palladium-catalyzed hydrogenation of the double bond, followed by substitution of the halogen for azide and final global deprotection yielded the isotope-code pair of azidosphingosines **22a** and **22b** ready for use in such quantitative chemical lipidomics studies.

Scheme 7.6 Overview of Delgado's (**22a**) work and this work (**22b**).

The synthesis of ¹³C₅-azido-sphingosine **22b** was accomplished following the strategy as detailed in Chapter 2^[12] and is outlined in Scheme 7.7.

Scheme 7.7 Synthesis of carbon-13-labeled azidosphinganine **22b**.

Reagents and conditions: (a) K¹³CN, EtOH/H₂O, 80 °C, 20 h, 99%; (b) NCS, PPh₃, THF, 20 h, 96%; (c) (i) DIBAL-H, THF, -78 °C, 1.5 h; (ii) 1 M HCl (aq), -78 °C, 30 min, 84%; (d) (i) ¹³C₂-HWE reagent, *n*-BuLi, 0 °C, 10 min; (ii) **26**, THF, 0 °C to r.t., 20 h, 89%; (e) Pt/C, H₂(g), EtOAc, r.t., 20 h, 93%; (f) (i) DIBAL-H, THF, -78 °C, 1.5 h; (ii) sat. Rochelle salt (aq), -78 °C to r.t.; (iii) ¹³C₂-HWE reagent, BuLi, 0 °C, 10 min; (iv) ¹³C₃-aldehyde, THF, 0 °C to r.t., 20 h, 94%; (g) (i) DIBAL-H, THF, -78 °C, 1.5 h; (ii) sat. Rochelle salt (aq), -78 °C to r.t.; (iii) PPh₃MeBr, NaH, THF, 80 °C, 3 h; (iv) ¹³C₅-aldehyde, THF, 0 °C to r.t., 20 h, 82%; (h) Grubbs 2nd cat., AcOH, DCM, 40 °C, 2 days, 86%; (i) PtO₂, H₂ (g), EtOAc, 20 h, 86%; (j) NaN₃, NaI, DMF, 55 °C, 20 h, 99%; (k) TFA, H₂O, 0 °C, 30 min, 71%.

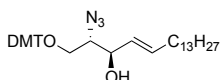
The isotope-encoded sphingolipids described in this thesis have been used for studying the metabolism of sphingolipids in tissue from healthy individuals as well as from patients suffering from various sphingolipidoses.^[14-19] Amongst others, the characteristically high

levels of lyso-GB3^[14] and glucosylsphingosine^[15] as secondary storage lipids in Fabry and Gaucher patients, respectively, can be quantified using the corresponding ¹³C₅-enriched sphingolipids as internal standard. By creating a comprehensive panel of stable isotope-enriched sphingolipids, and by developing the chemistry that allows expansion to sphingolipids not yet synthesized in the context of this Thesis, it is expected that the chemical toolset developed will find broader use in metabolomics studies, in relation to lysosomal storage disorders and perhaps as well in other human pathologies.

7.1 Experimental section

General Remarks: [¹³C₂]-acetic acid (99.95% isotopically pure, product code CLM-105), potassium [¹³C]-cyanide (99% isotopically pure, product code CLM-297), and [1,2,3-¹³C₃]-myristic acid (99% isotopically pure, product code CLM-3665) was purchased from Cambridge Isotope Laboratories, Inc., and was used as received. Commercially available reagents and solvents (Acros, Fluka, or Merck) were used as received, unless otherwise stated. CH₂Cl₂ and THF were freshly distilled before use, over P₂O₅ and Na/benzophenone, respectively. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide. Traces of water were removed from starting compounds by coevaporation with toluene. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out using forced flow of the indicated solvent systems on Screening Devices silica gel 60 (40–63 µm mesh). Size-exclusion chromatography was carried out on Sephadex LH20 (MeOH/CH₂Cl₂, 1:1). Analytical TLC was carried out on aluminum sheets (Merck, silica gel 60, F254). Compounds were visualized by UV absorption (254 nm), or by spraying with ammonium molybdate/cerium sulphate solution [(NH₄)₆Mo₇O₂₄· 4 H₂O (25 g/L), (NH₄)₄Ce(SO₄)₆· 2 H₂O (10 g/L), 10 % sulphuric acid in ethanol] or phosphormolybdic acid in EtOH (150 g/L), followed by charring (ca. 150 °C). IR spectra were recorded with a Shimadzu FTIR-8300 instrument and are reported in cm⁻¹. Optical rotations were measured with a Propol automatic polarimeter (sodium D-line, λ = 589 nm). ¹H and ¹³C NMR spectra were recorded with a Bruker AV 400 MHz spectrometer at 400.2 (¹H) and 100.6 (¹³C) MHz, or with a Bruker AV 600 MHz spectrometer at 600.0 (¹H) and 151.1 (¹³C) MHz. Chemical shifts are reported as δ values (ppm), and were referenced to tetramethylsilane (δ = 0.00 ppm) directly in CDCl₃, or using the residual solvent peak (D₂O). Coupling constants (*J*) are given in Hz, and all ¹³C spectra were proton decoupled. NMR assignments were made using COSY and HSQC, and in some cases TOCSY experiments. LC–MS analysis was carried out with an LCQ Advantage Max (Thermo Finnigan) instrument equipped with a Gemini C18 column (Phenomenex, 50 × 4.6 mm, 3 µm), using the following buffers: A: H₂O, B: acetonitrile, and C: aq. TFA (1.0 %). HPLC–MS purifications were carried out with an Agilent Technologies 1200 Series automated HPLC system with a Quadrupole MS 6130, equipped with a semi-preparative Gemini C18 column (Phenomenex, 250 × 10.00, 5 µm). Products were eluted using the following buffers: A: aq. TFA (0.2 %), B: acetonitrile (HPLC-grade), 5 mL/min. Purified products were lyophilized with a CHRIST ALPHA 2–4 LDPLUS apparatus to remove water and traces of buffer salts.

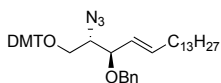
(2S, 3S, 4E)-2-Azido-1-(DMT)octadec-4-ene-3-ol (2). Azidosphingosine **1** (5.2 g, 16.0 mmol, 1.0 eq) was dissolved



in dry DCM (80 mL). The solution was cooled to 0 °C followed by addition of 2,4,6-collidine (4.7 mL, 35.2 mmol, 2.2. eq) and DMT-Cl (5.9 g, 17.6 mmol, 1.1 eq) and the reaction was stirred for 2 hours allowing to reach room temperature. The reaction was washed with sat. NaHCO₃ (50 mL) and brine (50 mL). The aqueous layers were extracted with DCM (50 mL) and the combined organic layers were dried (MgSO₄), filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (5% acetone, 1% Et₃N in pentane) giving **2** as a colorless oil (9.3 g, 14.7 mmol, 92%). R_f = 0.23 (5% Acetone in Pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.44 (d, 2 H, *J* = 7.6 Hz, H_{arom}), 7.32–7.19 (m, 7 H, H_{arom}), 6.84 (d, 1 H, *J* = 8.8 Hz, H_{arom}), 5.66 (dt, 1 H, *J* = 15.2, 6.8 Hz, H-5), 5.33 (dd, 1 H, *J* = 15.2, 7.2 Hz H-4), 4.19 (m, 1 H, H-3), 3.79 (s, 6 H, OMe_{DMT}), 3.52 (m, 1 H, H-2), 3.29 (d, 2 H, *J* = 5.6 Hz, H-1), 2.71 (bs, 1

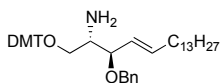
H, OH), 1.96 (q, 2 H, $J = 6.4$ Hz, H-6), 1.30-1.12 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, $J = 7.2$ Hz, H-18); ^{13}C NMR (101 MHz, CDCl_3) δ 158.66, 144.55, 135.76, 135.73 (4x $\text{C}_{\text{q-DMT}}$), 135.45 (C-5), 130.11, 130.01, 128.57, 128.17, 128.05, 127.78 (CH_{arom}), 127.03 (C-4), 113.31 ($\text{CH}_{\text{arom-DMT}}$), 86.97 ($\text{C}_{\text{q-DMT}}$), 73.25 (C-3), 65.89 (C-4), 63.38 (C-1), 55.33 (OMe_{DMT}), 32.38 (C-6), 32.06, 29.82, 29.79, 29.74, 29.59, 29.50, 29.31, 29.04, 22.83 (11x CH_2 C-7 to C-17), 14.27 (C-18); IR (neat) 3415, 2924, 2853, 2101, 1732, 1453, 1270, 1069 cm^{-1} ; HRMS calculated for $[\text{C}_{39}\text{H}_{53}\text{N}_3\text{O}_4 + \text{H}]^+$: 628.4116, found 628.4119.

(2S, 3S, 4E)-2-Azido-1-(DMT)octadec-4-ene-3-O-benzyl-ol (3). DMT-protected sphingosine **2** (4.7 g, 7.5 mmol, 1.0 eq) was dissolved in dry DMF (40 mL) under an atmosphere of argon. The solution was cooled to 0 °C and NaH (0.45 g, 11.3 mmol, 1.5 eq) was added and stirred for 30 minutes. To the reaction mixture was added benzyl bromide (1.4 mmol, 12.0 mmol, 1.6 eq) and was left to stir overnight. The reaction mixture was quenched

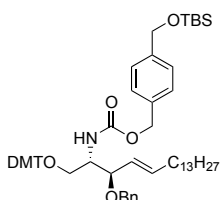


with MeOH (1 mL) and diluted with diethyl ether. The mixture was washed with water and brine. The water layers were extracted with diethyl ether and the combined organic layers were extracted with diethyl ether, dried (MgSO_4), filtered and concentrated *in vacuo*. The crude product was purified by silica column chromatography (pentane to 2% EtOAc in Pentane) giving a colorless oil giving a colorless oil (4.2 g, 6.3 mmol, 88%). $R_f = 0.4$ (2% EtOAc in Pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.44 (d, 2 H, $J = 7.6$ Hz, $\text{H}_{\text{arom-DMT}}$), 7.31-7.24 (m, 12H, H_{arom}), 6.80 (d, 4 H, $J = 8.8$ Hz, H_{arom}), 5.65 (dt, 1 H, $J = 15.6, 8.8$ Hz, H-5), 5.33 (dd, 1 H, $J = 15.6, 8.8$ Hz, H-4), 4.55 (d, 1 H, $J = 12.0$ Hz, $\text{CH}_{2\text{a-Bn}}$), 4.27 (d, 1 H, $J = 12.0$ Hz, $\text{CH}_{2\text{b-Bn}}$), 3.88 (m, 1 H, H-3), 3.77 (s, 6 H, OMe_{DMT}), 3.57 (m, 1 H, H-2), 3.25 (m, 2 H, H-1), 2.03 (q, 2 H, $J = 6.8$ Hz, H-6), 1.34-1.25 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, $J = 7.2$ Hz, H-18); ^{13}C NMR (101 MHz, CDCl_3) δ 158.59, 144.85 (2x $\text{C}_{\text{q-DMT}}$), 138.29 ($\text{C}_{\text{q-Bn}}$), 137.95 (C-5), 136.05, 136.02 ($\text{C}_{\text{q-DMT}}$), 130.19, 130.16, 128.40, 128.27, 127.94, 127.65, 127.56, 126.89 (CH_{arom}), 126.15 (C-4), 113.23 ($\text{CH}_{\text{arom-DMT}}$), 86.57 ($\text{C}_{\text{q-DMT}}$), 79.53 (C-3), 69.98 ($\text{CH}_{2\text{-Bn}}$), 65.17 (C-2), 63.14 (C-1), 55.31 (OMe_{DMT}), 32.47 (C-6), 32.06, 29.83, 29.80, 29.78, 29.60, 29.50, 29.31, 29.16, 22.83 (11x CH_2 C-7 to C-17), 14.26 (C-18); HRMS calculated for $[\text{C}_{46}\text{H}_{59}\text{N}_3\text{O}_4 + \text{H}]^+$: 718.4586, found 718.4591.

(2S, 3S, 4E)-2-amino-1-(DMT)octadec-4-ene-3-O-benzyl-ol (4). The DMT-Bn-protected sphingosine **3** (4.6 g, 6.4 mmol, 1.0 eq) was dissolved in THF:H₂O (95:5, 40 mL). To the solution was added PMe_3 (1 M in THF, 13 mL, 13 mmol, 2.0 eq) and left to stir overnight. The reaction mixture was concentrated *in vacuo* giving crude sphingosine **4** as a colorless oil (4.29 g, 6.2 mmol, 97%); ^1H NMR (400 MHz, CDCl_3) δ 7.43-7.16 (m, 14 H, H_{arom}), 6.79 (m, 4 H, $\text{H}_{\text{arom-DMT}}$), 5.67 (dt, 1 H, $J = 15.6, 8.8$ Hz, H-5), 5.31 (dd, 1 H, $J = 15.2, 8.8$ Hz, H-4), 4.54 (d, 1 H, $J = 12.0$ Hz, $\text{CH}_{2\text{a-Bn}}$), 4.26 (d, 1 H, $J = 12.0$ Hz, $\text{CH}_{2\text{b-Bn}}$), 3.78 (m, 1 H, H-3), 3.75 (s, 6 H, OMe_{DMT}), 3.20 (m, 2 H, H-1), 3.09 (m, 1 H, H-2), 2.03 (m, 2 H, H-6), 1.37-1.26 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, $J = 7.2$ Hz, H-18); ^{13}C NMR (101 MHz, CDCl_3) δ 158.47, 145.29 (2x $\text{C}_{\text{q-DMT}}$), 138.83 ($\text{C}_{\text{q-Bn}}$), 137.29 (C-5), 136.43 ($\text{C}_{\text{q-DMT}}$), 130.22, 130.18, 129.25, 128.54, 128.50, 128.42, 128.31, 128.05, 127.87, 127.82, 127.73, 127.38, 127.21, 127.10, 127.03, 126.73 (CH_{arom}), 126.00 (C-4), 113.11 ($\text{CH}_{\text{arom-DMT}}$), 85.92 ($\text{C}_{\text{q-DMT}}$), 81.87 (C-3), 69.97 ($\text{CH}_{2\text{-Bn}}$), 64.94 (C-1), 55.24 (OMe_{DMT}), 54.97 (C-2), 32.52 (C-6), 32.04, 30.43, 29.81, 29.77, 29.59, 29.47, 29.35, 22.81 (CH_2 C-7 to C-17), 14.24 (C-18); HMRS calculated for $[\text{C}_{46}\text{H}_{61}\text{NO}_4 + \text{H}]^+$: 692.4681, found 692.4679.

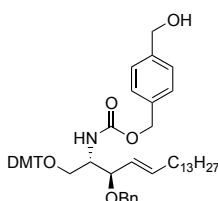


(2S, 3S, 4E)-2-N-(4-(OTBS)methyl)benzylcarbamate-1-(ODMT)-octadec-4-ene-3-O-benzyl-ol (6). Sphingosine **4** (4.1 g, 6.0 mmol, 1.0 eq) was dissolved in dry DMF (30 mL) under protected atmosphere. To the solution was DIPEA (1.35 mL, 7.8 mmol, 1.3 eq) added followed by addition of nitrophenol linker **5**^[4] (2.6 g, 6.3 mmol, 1.05 eq) at 0 °C. The reaction was stirred overnight allowing to reach room temperature. The reaction was diluted with diethyl ether and washed with water, twice with sat. aq. NaHCO_3 and brine. The water layers were extracted with diethyl ether and the combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (5% EtOAc in pentane) giving a colorless oil (4.3 g, 4.5 mmol, 75%). $R_f = 0.17$ (5% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.44 (d, 2 H, $J = 7.6$ Hz, $\text{H}_{\text{arom-DMT}}$), 7.30-



7.15 (m, 16 H, H_{arom}), 6.77-6.73 (m, 4 H, $H_{\text{arom-DMT}}$), 5.69 (dt, 1 H, $J = 15.2$, 8.0 Hz, H-5), 5.32 (dd, 1 H, $J = 15.2$, 8.0 Hz, H-4), 5.05 (s, 2 H, $\text{CH}_2\text{-Linker}$), 5.02 (d, 1 H, $J = 8.0$ Hz, NH), 4.73 (s, 2 H, $\text{CH}_2\text{-Linker}$), 4.56 (d, 1 H, $J = 12.0$ Hz, $\text{CH}_{2a\text{-Bn}}$), 4.28 (d, 1 H, $J = 12.0$ Hz, $\text{CH}_{2b\text{-Bn}}$), 4.03-3.94 (m, 2 H, H-2 and H-3), 3.73 (s, 6 H, OMe_{DMT}), 3.47 (m, 1 H, H-1a), 3.19 (dd, 1 H, $J = 9.2$, 3.6 Hz, H-1b), 2.02 (m, 2 H, H-6), 1.36-1.23 (m, 22 H, H-7 to H-17), 0.94 (s, 9 H, tBu_{TBS}), 0.88 (t, 3 H, $J = 7.2$ Hz, H-18), 0.10 (s, 6 H, Me_{TBS}); ^{13}C NMR (101 MHz, CDCl_3) δ 158.53 ($\text{C}_{\text{q-DMT}}$), 156.06 ($\text{C=O}_{\text{linker}}$), 145.02, 141.48, 138.55 (3x $\text{C}_{\text{q-arom}}$), 137.32 (C-5), 136.10, 135.31 (2x $\text{C}_{\text{q-arom}}$), 130.23, 130.12, 129.24, 128.35, 128.26, 128.09, 127.87, 127.69, 127.46, 127.32, 127.05, 126.81 (CH_{arom}), 126.23 (C-4), 126.10 (CH_{arom}), 133.10 ($\text{CH}_{\text{arom-DMT}}$), 86.08 ($\text{C}_{\text{q-DMT}}$), 80.17 (C-3), 70.29 ($\text{CH}_2\text{-Bn}$), 66.59 ($\text{CH}_2\text{-Linker}$), 64.79 ($\text{CH}_2\text{-Linker}$), 61.87 (C-1), 55.24 (OMe_{DMT}), 54.43 (C-2), 32.42 (C-6), 32.04, 29.83, 29.78, 29.64, 29.48, 29.37, 29.30 (CH_2 C-7 to C-17), 26.06 ($\text{CH}_3\text{-tBu-TBS}$), 22.81 (CH_2 C-7 to C-17), 18.51 ($\text{C}_{\text{q-tBu-TBS}}$), 14.25 (C-18), -5.15 (Me_{TBS}); HRMS calculated for $[\text{C}_{61}\text{H}_{83}\text{NO}_7\text{Si} + \text{H}]^+$: 970.6019, found 970.6023.

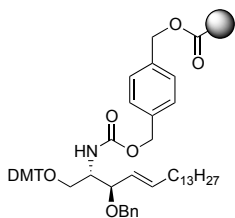
(2S, 3S, 4E)-2-N-(4-(hydroxymethyl)benzyl)carbamate-1-ol-octadec-4-ene-3-O-benzyl-ol (7). Protected



sphingosine-linker **6** (1.94 g, 2.0 mmol, 1.0 eq) was dissolved in THF (10 mL) and cooled to 0 °C. To the solution was added TBAF (1 M in THF, 3.0 mL, 3.0 mmol, 1.5 eq) and the reaction was stirred for 2 hours allowing to reach room temperature. The mixture was diluted with EtOAc and washed with water and brine. The water layers were extracted with EtOAc and combined organic layers were dried (MgSO_4), filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (5-10% EtOAc in pentane) giving a colorless oil (1.68 g, 1.96 mmol, 98 %). $R_f = 0.30$ (10% EtOAc in pentane); ^1H NMR (400 MHz, CDCl_3) δ 7.40-7.17 (m, 18 H, H_{arom}), 6.72 (d, $J = 8.8$ Hz, $H_{\text{arom-DMT}}$), 5.69 (dt, 1 H, $J = 11.6$, 8.4 Hz, H-5), 5.26 (dd, 1 H, $J = 11.6$, 8.4 Hz, H-4), 5.12-4.97 (m, 3 H, $\text{CH}_2\text{-Linker}$ and NH), 4.66 (s, 2 H, $\text{CH}_2\text{-linker}$), 4.56 (d, 1 H, $J = 11.6$ Hz, $\text{CH}_{2a\text{-Bn}}$), 4.27 (d, 1 H, $J = 11.6$ Hz, $\text{CH}_{2\text{-Bn}}$), 4.01-3.95 (m, 2 H, H-2 and H-3), 3.74 (d, 6 H, $J = 3.6$ Hz, OMe_{DMT}), 3.45 (m, 1 H, H-1a), 3.20 (m, 1 H, H-1b), 2.02 (m, 2 H, H-6), 1.42-1.26 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, $J = 7.2$ Hz, H-18); ^{13}C NMR (101 MHz, CDCl_3) δ 158.44 ($\text{C}_{\text{q-DMT}}$), 156.00 ($\text{C=O}_{\text{linker}}$), 145.01, 140.97, 138.49 ($\text{C}_{\text{q-arom}}$), 137.06 (C-5), 136.15 ($\text{C}_{\text{q-arom}}$), 130.16, 129.24, 128.82, 128.62, 128.41, 128.35, 128.26, 127.93, 127.86, 127.70, 127.48, 127.27, 127.21, 127.18 (CH_{arom} and C-4), 113.14 ($\text{CH}_{\text{arom-DMT}}$), 86.06 ($\text{C}_{\text{q-DMT}}$), 80.10 (C-3), 70.30 ($\text{CH}_2\text{-Bn}$), 66.39 ($\text{CH}_2\text{-linker}$), 65.04 ($\text{CH}_2\text{-linker}$), 61.87 (C-1), 55.26 (OMe_{DMT}), 54.46 (C-2), 32.43, 32.06, 29.82, 29.78, 29.63, 29.57, 29.47, 29.36, 29.29, 29.17, 22.80 (C-6 to C-17), 14.25 (C-18); HRMS calculated for $[\text{C}_{55}\text{H}_{69}\text{NO}_7 + \text{H}]^+$: 856.5154, found 856.5149.

Down tuning of loading resin. Carboxypolystyryl resin (~2.2 mmol, 1 g) was swelled with DCM (5 mL) and purged with argon for 15 minutes. The solvent was released and the resin washed with 3x with DCM, followed by three times swelling and shrinking with DCM/Hexane. After this the resin was washed again three times with DMC and then left to dry. The resin was swollen by THF (5 mL) for 15 minutes before addition of MeOH (0.15 mL, 3.8 mmol, 2.25 eq). This suspension was shaken for 15 minutes before trimethylsilyl diazomethane (2 M in hexane, 0.84 mL, 1.7 mmol, 0.75 eq) was added. The suspension was shaken overnight. It was finally washed with 3x DCM, 3x swelling/shrinking with DCM/hexane and 3x washed with THF.

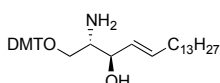
Coupling basic cleavable sphingosine linker to resin (8). The resin was swollen with DCM (5 mL) for 15 minutes,



followed by filtration. To the swollen resin DCM (9 mL) was added followed by addition of a solution existing of sphingosine linker **7** (1.4 g, 1.65 mmol, 3.0 eq) and DIC (0.26 mL, 1.65 mmol, 3.0 eq) in DCM (4 mL). Next, DMAP (10 mg, 0.05 mmol, 0.1 eq) was added and the reaction was left to shake overnight. The reaction was quenched by addition of MeOH (0.15 mL) and left to shake for 1 hour. The resin was filtered and washed three times with DCM, followed by swelling/shrinking DCM/hexane procedure. Finally, the resin was washed with three times with DCM and then dried.

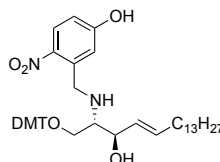
Removal of DMT-group and determination of resin loading (9). This procedure was performed in triple to get accurate numbers. DMT-Linker (10 mg) was suspended in 3% TCA in DCM (3 mL) and shaken for 5 minutes (the mixture turned instantly to orange/red). The suspension was filtered and diluted with DCM to 10 mL. From this 10 mL DMT solution, 0.1 mL was taken and diluted to 10 mL with DCM. Absorption of the dilution was measured and the resin loading derived from the obtained values using the following formula: Loading ($[A_{504}]/76$) \times 100 = loading in mmol/g.

(2S, 3S, 4E)-2-Amino-1-(DMT)octadec-4-ene-3-ol (10). Protected azidosphingosine **2** (1.0 g, 1.6 mmol, 1 eq) was



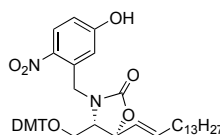
dissolved in EtOH (8 mL). The solution was purged with argon before the addition of Lindlar catalyst (5% wt. Pd on CaCO₃, poisoned with lead). The reaction mixture was purged with H₂ (g) for 15 minutes. The reaction was stirred overnight under an atmosphere of hydrogen gas. The mixture was filtered over Celite and concentrated *in vacuo*. The crude product was purified by silica column chromatography (2% MeOH, 1% Et₃N in DCM) giving a colorless oil (0.54 g, 0.90 mmol, 54%). R_f = 0.5 (5% MeOH in DCM); ¹H NMR (400 MHz, CDCl₃) δ 7.41 (d, 2 H, J = 7.6 Hz, H_{arom-DMT}), 7.31-7.18 (m, 7 H, H_{arom-DMT}), 6.82 (d, 4 H, J = 7.2 Hz, H_{arom-DMT}), 5.64 (dt, 1 H, J = 15.2, 7.6 Hz, H-5), 5.26 (dd, 1 H, J = 15.2, 6.8 Hz, H-4), 4.12 (t, 1 H, J = 6.0 Hz, H-3), 3.78 (s, 6 H, OMe_{DMT}), 3.18 (d, 2 H, J = 5.6 Hz, H-1), 3.02 (m, 1 H, H-2), 2.42 (bs, 2 H, NH₂), 1.94 (m, 2 H, H-6), 1.34-1.23 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, J = 7.2 Hz, H-18); ¹³C NMR (101 MHz, CDCl₃) δ 158.58, 144.90, 136.13, 136.09 (C_{q-DMT}), 134.16 (C-5), 130.11, 129.24, 128.79, 128.20, 128.17, 127.88, 127.74 (CH_{arom-DMT}), 126.91 (C-4), 113.26 (CH_{arom-DMT}), 86.43 (C_{q-DMT}), 74.84 (C-3), 65.81 (C-1), 55.30 (OMe_{DMT}), 46.29 (C-2), 32.04 (C-6), 29.82, 29.78, 29.65, 29.61, 29.48, 29.37, 29.27, 22.81 (CH₂ C-7 to C-17), 14.24 (C-18); IR (neat) 3330, 2914, 2849, 1661, 1469, 1198 cm⁻¹; HRMS calculated for [C₃₉H₅₅NO₄ + H]⁺: 602.4211, found 602.4223.

(2S, 3S, 4E)-2-N-(5-hydroxy-2-nitrobenzyl)-1-(DMT)octadec-4-ene-3-ol (11). The DMT protected sphingosine **10**



(0.5 g, 0.83 mmol, 1 eq) was dissolved in wet THF (3 mL). 5-hydroxy-2-nitrobenzaldehyde (0.13 g, 0.83 mmol, 1.0 eq) was added and stirred for one hour. To the reaction was added NaBH₃CN (52 mg, 0.83 mmol, 1.0 eq) was added and mixture was stirred overnight at room temperature. The reaction was quenched with NaHCO₃ (10 mL) and EtOAc (10 mL). The two layers were separated and the water layer was extracted with EtOAc. The combined organic layers were washed with brine, dried with MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (20% EtOAc in Pentane) giving a yellow oil (0.38 g, 0.5 mmol, 62%) NMR will depend on acidity of the chloroform. R_f = 0.27 (30% EtOAc in Pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.93 (d, 1 H, J = 9.2 Hz, H_{arom-nitro phenol}), 7.38 (d, 2 H, J = 7.2 Hz, H_{arom-DMT}), 7.29-7.14 (m, 7 H, H_{arom-DMT}), 6.82-6.79 (m, 5 H, 4x H_{arom-DMT} and H_{arom-nitro phenol}), 6.58 (dd, 1 H, J = 9.2, 2.0 Hz, H_{arom-nitro phenol}), 5.65 (dt, 1 H, J = 15.6, 7.6 Hz, H-5), 5.20 (dd, 1 H, J = 15.2, 6.0 Hz, H-4), 4.35 (m, 1 H, H-3), 4.06 (d, 1 H, J = 14.0 Hz, CH_{2a-nitrophenol}), 3.94 (d, 1 H, J = 14.0 Hz, CH_{2b-nitrophenol}), 3.73 (s, 6 H, OMe_{DMT}), 3.41 (m, 2 H, H-1), 2.91 (m, 1 H, H-2), 1.91 (m, 2 H, H-6), 1.33-1.22 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, J = 6.8 Hz, H-18); ¹³C NMR (101 MHz, CDCl₃) δ 165.43 (C_{q-nitro phenol}), 158.78, 144.35 (C_{q-arom-DMT}), 139.38 (C-5), 139.09 (C_{q-nitro phenol}), 135.41, 135.26 (C_{q-DMT}), 130.15, 130.13, 129.26, 128.80, 128.20, 127.24, (CH_{arom-DMT} and CH_{nitrophenol}), 125.34 (C-4), 115.50, 114.38 (CH_{nitrophenol}), 133.48 (CH_{arom-DMT}), 78.52 (C-3), 62.48 (C-2), 59.59 (C-1), 55.34 (OMe_{DMT}), 46.21 (CH_{2-nitrophenol}), 32.48, 32.05, 29.83, 29.80, 29.62, 29.50, 29.41, 29.09, 22.82 (C-6 to C-17), 14.29 (C-18); HRMS calculated for [C₄₆H₆₀N₂O₇ + H]⁺: 753.4481, found 753.4494.

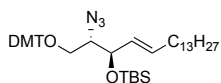
(2S, 3S, 4E)-2,3-O,N-carbamate-2-N-(5-hydroxy-2-nitrobenzyl)-1-(DMT)octadec-4-ene (12). The nitro phenol



sphingosine **11** (0.18 g, 0.4 mmol, 1.0 eq) was dissolved in dry DCM. To the solution was added carbonyl-diimidazole (1.23 g, 7.6 mmol, 2 eq) and was stirred overnight at room temperature. The mixture was concentrated and purified by silica column chromatography (10% EtOAc in Pentane) giving a yellow oil (0.17 g 0.36 mmol, 90%). R_f = 0.45 (20% EtOAc in Pentane); ¹H NMR (400 MHz, CDCl₃) δ 8.03 (d, 1 H, J = 9.2 Hz,

H_{arom}-nitro phenol), 7.40-7.21 (m, 9 H, H_{arom}-DMT), 6.88-6.81 (m, 5 H, 4x H_{arom}-DMT and H_{arom}-nitro phenol), 6.70 (dd, 1 H, *J* = 8.8, 2.4 Hz, H_{arom}-nitro phenol), 5.81 (dt, 1 H, *J* = 15.6, 8.4 Hz, H-5), 5.52 (dd, 1 H, *J* = 15.2, 8.4 Hz, H-4), 5.21 (t, 1 H, *J* = 8.4 Hz, H-3), 5.02 (d, 1 H, *J* = 18.4 Hz, CH_{2a}-nitrophenol), 4.57 (d, 1 H, *J* = 17.6 Hz, CH₂-nitrophenol), 3.81-3.74 (m, 7 H, OMe_{DMT} and H-2), 3.55 (dd, 1 H, *J* = 10.8, 3.2 Hz, H-1_a), 3.13 (dd, 1 H, *J* = 10.8, 2.4 Hz, H-1_b), 1.88 (m, 2 H, H-6), 1.34-1.19 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18); ¹³C NMR (101 MHz, CDCl₃) δ 163.09 (C_q-nitrophenol), 158.80, 144.00 (C_q-nitrophenol), 140.67 (C-5), 135.21, 135.06 (C_q-DMT), 130.15, 130.03, 129.75, 129.34, 128.80, 128.20, 127.24 (CH_{arom}-DMT and CH_{arom}-nitro phenol), 122.25 (C-4), 115.56 114.31 (CH_{arom}-nitrophenol), 113.48, 113.42 (CH_{arom}-DMT), 79.78 (C-3), 60.88 (C-2), 59.69 (C-1), 55.37 (OMe_{DMT}), 43.96 (CH₂-nitrophenol), 32.46, 32.09, 29.89, 29.70, 29.53, 29.47, 29.03, 22.85 (C-6 to C-17), 14.29 (C-18); IR (neat); HRMS calculated for [C₄₇H₅₈N₂O₈ + H]⁺: 779.4273, found 779.4278.

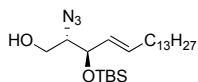
(2S, 3S, 4E)-2-Azido-1-(DMT)octadec-4-ene-3-O-TBS. (13).



1-DMT-sphingosine **2** (1.25g, 2 mmol, 1.0 eq) was dissolved in dry DCM (10 mL) under protected atmosphere and cooled to 0 °C. 2,4,6-Collidine (0.65 mL, 5 mmol, 2.5 eq) was added to the solution followed by addition of TBSOTf (0.57 mL, 2.5 mmol, 1.25 eq). The reaction was stirred overnight at 4 °C. The reaction was diluted with DCM and washed with sat. aq. NaHCO₃ and brine. The

water layers were extracted with DCM and the combined organic layers were dried (MgSO₄), filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (1% to 5% Acetone in Pentane) giving a colorless oil (0.93 g, 1.3 mmol, 65%). *R*_f = 0.6 (5% Acetone in pentane); ¹H NMR (400 MHz, CDCl₃) δ 7.43 (d, 2 H, *J* = 7.6 Hz, H_{arom}-DMT), 7.33-7.29 (m, 12 H, H_{arom}), 6.81 (dd, 4 H, *J* = 8.8, 1.6 Hz, H_{arom}), 5.52 (dt, 1 H, *J* = 15.2, 8.8 Hz, H-5), 5.32 (dd, 1 H, *J* = 15.2, 7.2 Hz, H-4), 4.07 (m, 1 H, H-3), 3.78 (s, 6 H, OMe_{DMT}), 3.54 (m, 1 H, H-2), 3.15 (m, 2 H, H-1), 2.03 (m, 2 H, H-6), 1.34-1.24 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18), 0.78 (s, 9 H, TBS_{tBu}), -0.05 (s, 3 H, TBS_{Me}), -0.07 (s, 3 H, TBS_{Me}); ¹³C NMR (101 MHz, CDCl₃) δ 158.60, 144.89, 136.19, 136.06 (2x C_q-DMT), 134.34 (C-5), 130.16, 130.10, 129.00, 128.26, 127.93 127.73 (CH_{arom}-DMT), 126.80 (C-4), 113.23 (CH_{arom}-DMT), 86.57 (C_q-DMT), 74.52 (C-3), 67.44 (C-2), 63.45 (C-1), 55.31 (OMe_{DMT}), 32.31, 32.06, 29.83, 29.81, 29.76, 29.60, 29.49, 29.31, 29.15, (11x CH₂ C-6 to C-17), 25.83 (TBS_{tBu}), 22.82 (CH₂ C-6 to C17), 18.09 (TBS_{q-tBu}) 14.25 (C-18), -3.98, -4.89 (2x TBS_{Me}).

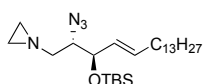
(2S, 3S, 4E)-2-Azido-1-octadec-4-ene-3-O-TBS. (14).



Silylated sphingosine **13** (0.9 g, 1.22 mmol, 1.0 eq) was dissolved in dry DCM (12 mL) under protected atmosphere and dodecanethiol (0.6 mL, 2.5 mmol, 2.0 eq) was added to solution. The solution was cooled to 0 °C, followed by addition of trifluoroacetic acid (72 μL, 0.96 mmol, 0.8 eq) and the reaction was left to stir for 2 hours. The reaction was diluted with DCM and was washed with sat. aq. NaHCO₃

and brine. The water layers were extracted with DCM and the combined organic layers were dried (MgSO₄), filtered and concentrated *in vacuo*. The product was purified by silica column chromatography giving a colorless oil (0.42 g, 0.96 mmol, 80%). *R*_f = 0.15 (5% Acetone in pentane); ¹H NMR (400 MHz, CDCl₃) δ 5.61 (dt, 1 H, *J* = 15.6, 7.2 Hz, H-5), 5.40 (dd, 1 H, *J* = 15.6, 7.2 Hz, H-4), 4.17 (m, 1 H, H-3), 3.66-3.62 (m, 2 H, H-1), 3.36 (m, 1 H, H-2), 2.20 (bs, 1 H, OH), 2.00 (m, 2 H, H-6), 1.34 (m, 2 H, H-7), 1.30-1.20 (m, 20 H, H-8 to H-17), 0.89-0.81 (m, 12 H, H-18 and TBS_{tBu}), 0.04 (s, 3 H, TBS_{Me}), -0.01 (s, 3 H, TBS_{Me}); ¹³C NMR (101 MHz, CDCl₃) δ 134.92 (C-5), 129.14 (C-4), 75.39 (C-3), 67.83 (C-2), 62.37 (C-1), 32.38, 32.27, 32.07, 29.84, 29.82, 29.75, 29.60, 29.33, 29.10, 28.80 (11x CH₂ C-6 to C-17), 25.87 (TBS_{tBu}), 22.82 (CH₂ C-6 to C17), 18.16 (TBS_{q-tBu}) 14.25 (C-18), -3.96, -4.88 (2x TBS_{Me}).

(2S, 3S, 4E)-2-Azido-1-(ethylenimine)-octadec-4-ene-3-O-TBS. (15).



sphingosine **14** (0.4 g, 0.91 mmol, 1.0 eq) was dissolved in dry DCM (5 mL) under protected atmosphere and cooled to 0 °C. Pyridine (88 μL, 1.1 mmol, 1.2 eq) was added followed by addition of Tf₂O (0.18 mL, 1.1 mmol, 1.2 eq). The mixture was stirred for 1 hour following dilution with DCM (10 mL).

The reaction was washed with water and brine. The water layers were extracted with DCM and the combined organic layers were dried (MgSO₄), filtered and concentrated *in vacuo* giving the crude triflate, which directly used without any further purification in the next step. The crude triflated sphingosine was dissolved in dry DCM (5 mL) under protected atmosphere and cooled to -20 °C. DIPEA (0.19 mL, 1.1 mmol, 1.2

eq) was added followed by addition of dry ethyleneimine (1 mL 20 mmol, 20 eq), and was left stirring for 3 hours. The reaction mixture was quenched with MeOH (0.1 mL) and washed with water and brine. The water layers were extracted with DCM and combined organic layers were dried (MgSO₄) filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (pentane to 2% acetone in pentane) giving a colorless oil (0.18 g, 0.40 mmol 45%). [α]_D²⁰: -26 (C= 0.5, CHCl₃); R_f = 0.72 (5% acetone in pentane); ¹H NMR (400 MHz, CDCl₃) δ 5.64 (dt, 1 H, *J* = 15.2, 7.6 Hz, H-5), 5.42 (dd, 1 H, *J* = 15.6, 7.2 Hz, H-4), 4.19 (m, 1 H, H-3), 3.54 (m, 1 H, H-2), 2.36 (dd, 1 H, *J* = 12.8, 4.0 Hz, H-1_a), 2.10 (dd, 1 H, *J* = 12.4, 8.4 Hz, H-1_b), 2.03 (q, 2 H, *J* = 7.2 Hz, H-6), 1.84 (dd, 1 H, *J* = 6.0, 4.0 Hz, H_{aziridine}), 1.75 (dd, 1 H, *J* = 6.0, 4.0 Hz, H_{aziridine}), 1.40-1.24 (m, 23 H, H-7 to H-17 and H_{aziridine}), 1.10 (dd, 1 H, *J* = 6.0, 4.0 Hz, H_{aziridine}), 0.91-0.86 (m, 12 H, H-18 and TBS_{tBu}), 0.06 (s, 3 H, TBS_{Me}), 0.03 (s, 3 H, TBS_{Me}); ¹³C NMR (101 MHz, CDCl₃) δ 134.56 (C-5), 128.86 (C-4), 75.53 (C-3), 67.92 (C-2), 61.23 (C-1), 32.36, 32.05, 29.81, 29.78, 29.76, 29.68, 29.60, 29.49, 29.15, 28.98, 28.23, 27.03, 26.01 (11x CH₂ C-6 to C-17 and 2x CH₂-aziridine), 25.90 (TBS_{tBu}), 18.21 (TBS_{Cq-tBu}), 14.25 (C-18), -3.98, -4.80 (2x TBS_{Me}).

[1-¹³C₁]-10-Hydroxydecanitrile (24). 9-Bromo-1-nonanol **23** (8.87 g, 39.8 mmol, 1.05 eq) was dissolved in EtOH/H₂O (9:1, 17 mL), and K¹³CN (2.5 g, 37.9 mmol) was added. The reaction mixture was heated at 80 °C overnight. The reaction was then diluted with ether and was washed 2x with water and 1x with brine. The water layers were extracted with ether and the combined organic layer were dried (Na₂SO₄) and concentrated *in vacuo*. The product was purified by column chromatography (20% EtOAc in petroleum ether) giving colorless oil (6.43 g, 37.8 mmol, 99%). R_f = 0.51 (1:1 EtOAc: petroleum ether). ¹H NMR (400 MHz, CDCl₃) δ 3.63 (t, 2 H, *J* = 6.8 Hz, H-10), 2.34 (dt, 2 H, *J* = 9.6, 7.2 Hz, H-2), 1.69-1.62 (m, 3 H, H-9 and OH), 1.55 (m, 2 H, H-3), 1.44 (m, 2 H, H-8), 1.39-1.28 (m, 8 H, H-4 to H-7). ¹³C NMR (101 MHz, CDCl₃) δ 119.9 (CN), 63.8 (C-OH), 32.7, 29.3 (d, *J* = 1.5 Hz), 28.71, 28.65 (d, *J* = 3.3 Hz) 25.7, 25.35 (d, *J* = 2.7 Hz), 17.1 (d, *J* = 56.6 Hz, C-2). IR (neat): 3294, 2926, 2854, 2193, 1463, 1425, 1055 cm⁻¹. HRMS calculated for [C₉¹³CH₁₉NO + H]⁺: 171.1466, found 171.1470.

[1-¹³C₁]-10-Chloro-decanitrile (25). [1-¹³C₁]-10-Hydroxydecanitrile **24** (6.21 g, 36.5 mmol) was dissolved in THF (240 mL) to which PPh₃ (10.5 g, 40.2 mmol, 1.1 eq) and NCS (5.36 g, 40.2 mmol, 1.1 eq) were added. The mixture was stirred overnight at room temperature. After the reaction was finished on TLC the mixture was diluted with EtOAc and 2x washed with water and 1x with brine. The water layers were extracted with EtOAc and the combined organic layers were dried (Na₂SO₄) and concentrated. Petroleum ether was added to residue and the remaining solids were filtered off and the organic solution was concentrated *in vacuo*. The product was purified by column chromatography (3% EtOAc in petroleum ether) giving a colorless oil (6.60 g, 35.0 mmol, 96%). R_f = 0.24 (3% EtOAc in petroleum ether). ¹H NMR (400 MHz, CDCl₃) δ 3.53 (t, 2 H, *J* = 6.8 Hz, H-10), 2.34 (dt, 2 H, *J* = 9.6, 7.2 Hz, H-2), 1.77 (p, 2 H, *J* = 6.9 Hz, H-9), 1.65 (m, 2 H, H-3), 1.48-1.37 (m, 4 H, H-4 and H-8), 1.34-1.25 (m, 6 H, H-5 to H-7). ¹³C NMR (101 MHz, CDCl₃) δ 119.7 (CN), 45.0 (C-10), 32.4 (C-9), 29.00, 28.59, 28.52, 28.46 (d, *J* = 3.3 Hz), 26.66 (5x -CH₂-, C-4 to C-8), 25.2 (d, *J* = 2.7 Hz, C-3), 17.0 (d, *J* = 55.5 Hz, C-2). IR (neat): 2928, 2857, 2193, 1462, 1427, 1307, 721, 648 cm⁻¹. HRMS calculated for [C₉¹³CH₁₈NCl + H]⁺: 189.1206, found 189.1208.

[1-¹³C₁]- 10-chlorodecanal (26). [1-¹³C₁]-10-Chloro-decanitrile **25** (2.09 g, 10.66 mmol) was dissolved in dry DCM (40 mL) and was cooled to -78 °C under protected atmosphere. DIBAL-H (1.5 M in toluene, 10.67 mL, 16.0 mmol, 1.5 eq) was slowly added, and stirred for 1.5 h at -78 °C. The reaction mixture was quenched with 1 M HCl (aq) (15 mL) and was stirred for 30 minutes at -78 °C. Then the reaction mixture was warmed to room temperature and was diluted with ether. The mixture was washed 2x with water and 1x with brine. The water layers were extracted with ether and the combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The product was purified by column chromatography (3% EtOAc in petroleum ether) giving a colorless oil (1.71 g, 8.95 mmol, 84 %). R_f = 0.25 (3% EtOAc in petroleum ether). ¹H NMR (400 MHz, CDCl₃) δ 9.76 (dt, 1 H, *J* = 169.6, 1.6 Hz, H-1), 3.52 (t, 2 H, *J* = 6.8 Hz, H-10), 2.43 (dq, 2 H, *J* = 7.2, 1.6 Hz, H-2), 1.78 (p, 2 H, *J* = 6.8 Hz, H-9), 1.63 (m, 2 H, H-3), 1.42 (m, 2 H, H-8), 1.41-1.27 (m, 8 H, H-4 to H-7). ¹³C

NMR (101 MHz, CDCl_3) δ 202.8 (t, J = 19.2 Hz, C-1), 45.1 (C-10), 43.9 (d, J = 39.4 Hz, C-2), 32.64 (C-9), 2x 29.27 (CH_2 C-4 to C-8), 29.12 (d, J = 3.4 Hz, C-3), 28.83, 26.86, 21.8 (3x CH_2 C-4 to C-8). IR (neat); 2926, 2855, 2710, 1664, 1464.

[1,2,3- $^{13}\text{C}_3$]-(*E*)-12-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide and [1,2,3- $^{13}\text{C}_3$]-(*Z*)-12-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide (27). Diethyl ([1,2- $^{13}\text{C}_2$]-*N*-methoxy-*N*-methylacetamide)phosphonate (2.46 g, 10.2 mmol, 1.05) was dissolved in dry THF (47 mL) under protected atmosphere. *n*-BuLi (1.6 M in hexane, 6.37 mL, 10.2 mmol, 1.05 eq) was added at 0 °C and stirred for 10 min at this temperature. [1- $^{13}\text{C}_1$]-10-chlorodecanal **26** (1.86 g, 9.71 mmol) was dissolved in dry THF (10.0 mL) was added to the phosphonate carbanion and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with ether and was washed 2x with water and 1x brine. The water layers were extracted with ether and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by column chromatography (10% EtOAc in petroleum ether) giving a colorless oil (2.41 g, 8.64 mmol, 89%, *E*:*Z* = 11:1). R_f (*E*) = 0.12 and R_f (*Z*) = 0.20 (10% EtOAc in petroleum ether). (*E*-isomer) ^1H NMR (400 MHz, CDCl_3) δ 6.97 (dm, 1 H, J = 154.0 Hz, H-3), 6.41 (ddd, 1 H, J = 160.4, 15.2, 3.6 Hz, H-2), 3.70 (s, 3 H, $\text{CH}_3\text{-OMe}$), 3.53 (t, 2 H, J = 6.4 Hz, H-12), 3.24 (s, 3 H, $\text{CH}_3\text{-NMe}$), 2.24 (m, 2 H, H-4), 1.76 (p, 2 H, J = 6.8 Hz, H-11), 1.52-1.38 (m, 4 H, H-5 and H-10), 1.37-1.20 (m, 8 H, H-6 to H-9). ^{13}C NMR (101 MHz, CDCl_3) δ 167.1 (d, J = 67.7 Hz, C-1), 148.0 (d, J = 71.7 Hz, C-3), 118.6 (dd, J = 71.7, 67.7 Hz, C-2), 61.7 ($\text{CH}_3\text{-OMe}$), 45.2 (C-12), 32.7 (C-11), 32.6 (m, C-4), 31.8 (m, $\text{CH}_3\text{-NMe}$), 29.41, 29.35, 29.3 (d, J = 3.6 Hz), 28.9, 28.35 (m), 26.9 (6x CH_2 C-5 to C-10). IR (neat) 2926, 2854, 1616, 1581, 1462, 1367, 1177, 983 cm^{-1} . (*Z*-isomer) ^1H NMR (400 MHz, CDCl_3) δ 6.24 (dd, 1 H, J = 162.0, 10.4 Hz, H-2), 6.11 (dm, 1 H, J = 152.4 Hz, H-3), 3.68 (s, 3 H, $\text{CH}_3\text{-OMe}$), 3.53 (t, 2 H, J = 6.8 Hz, H-12), 3.21 (s, 3 H, $\text{CH}_3\text{-NMe}$), 2.61 (m, 2 H, H-4), 1.76 (p, 2 H, J = 7.0 Hz, H-11), 1.49-1.37 (m, 4 H, H-5 and H-10), 1.36-1.22 (m, 8 H, H-6 to H-9). ^{13}C NMR (101 MHz, CDCl_3) δ 167.6 (d, J = 66.7 Hz, C-1), 147.7 (d, J = 71.7 Hz, C-3), 117.9 (t, J = 69.7 Hz, C-2), 61.5 ($\text{CH}_3\text{-OMe}$), 45.2 (C-12), 32.63 (C-11), 32.2-31.8 (m, $\text{CH}_3\text{-NMe}$ and C-4), 29.45, 29.31, 2x 29.4-29.2 (m), 28.9, 26.94 (6x CH_2 C-5 to C-10). IR (neat); 2926, 2855, 1612, 1460, 1332, 1177, 997 cm^{-1} . HRMS calculated for $[\text{C}_{11}^{13}\text{C}_3\text{H}_{26}\text{NO}_2\text{Cl} + \text{H}]^+$: 279.1652, found 279.1651.

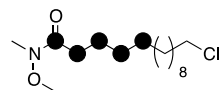
[1,2,3- $^{13}\text{C}_3$]-12-Chloro-*N*-methoxy-*N*-methyl-dodecanamide (28). [1,2,3- $^{13}\text{C}_3$]-(*E/Z*)-12-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide **27** (5.76 g, 20.68 mmol) was dissolved in EtOAc (240 mL). The solution was purged with argon under stirring and a catalytic amount of palladium 10% on charcoal (1.0 g, 1.0 mmol, 0.05 eq) was added. The reaction was stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere overnight.

The palladium was removed by filtration over a plug of Celite and then rinsed with EtOAc followed by removal of the solvent under reduced pressure giving the desired product, a colorless oil (5.40 g, 19.3 mmol, 93%). R_f = 0.15 (10% EtOAc in petroleum ether). ^1H NMR (400 MHz, CDCl_3) δ 3.68 (s, 3 H, $\text{CH}_3\text{-OMe}$), 3.53 (t, 2 H, J = 6.8 Hz, H-12), 3.18 (s, 3 H, $\text{CH}_3\text{-NMe}$), 2.41 (dm, 2 H, J = 127.6 Hz, H-2), 1.82-1.72 (m, 2 H, H-11), 1.64 (dm, 2 H, J = 127.6 Hz, H-3), 1.54-1.38 (m, 2 H, H-10), 1.37-1.22 (m, 12 H-4 to H-9). ^{13}C NMR (101 MHz, CDCl_3) δ 174.9 (d, J = 51.5 Hz, C-1), 61.3 ($\text{CH}_3\text{-OMe}$), 45.3 (C-12), 32.64 (C-11), 32.2 (dd, J = 51.5, 37.2 Hz, C-2 and $\text{CH}_3\text{-NMe}$), 29.48 (m, C-4), 28.87, 26.85, 24.64 (d, J = 35.1 Hz, C-3), 24.7 (7x CH_2 C-4 to C-10). IR (neat); 2924, 2852, 1626, 1464, 1371 1175, 1001 cm^{-1} . HRMS calculated for $[\text{C}_{11}^{13}\text{C}_3\text{H}_{26}\text{NO}_2\text{Cl} + \text{H}]^+$: 281.1808, found 281.1805.

[1,2,3,4,5- $^{13}\text{C}_5$]-(*E*)-14-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide and [1,2,3,4,5- $^{13}\text{C}_5$]-(*Z*)-14-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide (29). [1,2,3- $^{13}\text{C}_3$]-12-Chloro-*N*-Methoxy-*N*-methyl-dodecanamide **28** (5.29 g, 18.6 mmol) was dissolved in dry THF (75 mL) and was cooled to -78°C under protected atmosphere. DIBAL-H (1.5 M in toluene, 14.9 mL, 22.4 mmol, 1.2 eq) was added and the mixture was stirred for 1 hour at -78°C. The mixture was quenched with sat. Rochelle salt (aq) (75 mL) at -78°C and was roughly stirred to room temperature. The mixture was diluted with ether and was washed 2x with water and 1 x with brine. The water layers were extracted with ether and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* giving crude [1,2,3- $^{13}\text{C}_3$]-12-Chloro-dodecanal as a clear oil which was used without further purification. [1,2- $^{13}\text{C}_2$]-

Diethyl (*N*-Methoxy-*N*-methylacetamide)phosphonate (4.33 g, 18.0 mmol, 1.05) was dissolved in dry THF (90 mL) under protected atmosphere. *n*-BuLi (1.6 M in hexane, 11.2 mL, 18.0 mmol, 1.05 eq) was added at 0 °C and stirred for 10 min at this temperature. 12-Chloro-dodecanal (3.80 g, 17.1 mmol) was dissolved in dry THF (18 mL) was added to the phosphonate carbanion and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with ether and was washed 2x with water and 1x with brine. The water layers were extracted with ether and the combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The product was purified by column chromatography (10% EtOAc in petroleum ether) giving a colorless oil (5.41 g, 17.5 mmol, 94%, E:Z = 10.5: 1). *R_f* (E) = 0.2 and *R_f* (Z) = 0.31 (10 % EtOAc in petroleum ether). (**E-isomer**) ¹H NMR (400 MHz, CDCl₃) δ 6.96 (dm, 1 H, *J* = 156.0 Hz, H-3), 6.38 (ddm, 1 H, *J* = 160.4, 15.6 Hz, H-2), 3.70 (s, 3 H, CH₃-OMe), 3.53 (t, 2 H, *J* = 6.8 Hz, H-14), 3.24 (s, 3 H, CH₃-NMe), 2.21 (dm, 2 H, *J* = 127.2 Hz, H-4), 1.76 (p, 2 H *J* = 7.0 Hz, H-13), 1.68-1.20 (m, H 16, H-5 to H-12). ¹³C NMR (101 MHz, CDCl₃) δ 167.2 (dd, *J* = 67.1, 5.7 Hz, C-1), 148.1 (dd, *J* = 71.7, 41.2 Hz, C-3), 118.7 (ddd, *J* = 71.7, 67.1, 4.6 Hz, C-2), 61.8 (CH₃-OMe), 45.3 (C-14), 32.5 (C-13), 32.4 (dddd, *J* = 41.2, 33.8, 5.7, 1.3 Hz, C-4), 32.2 (CH₃-NMe), 29.6-29.0 (6x CH₂ C-6 to C-11), 28.6 (dd, *J* = 36.6, 33.8 Hz, C-5), 27.0 (C-12). IR (neat); 2922, 2852, 1616, 1581, 1462, 1368, 1177, 988 cm⁻¹. (**Z-isomer**) ¹H NMR (400 MHz, CDCl₃) δ 6.22 (dm, 1 H, *J* = 161.2 Hz, H-2), 6.11 (dm, 1 H, *J* = 152.8 Hz, H-3), 3.68 (s, 3 H, CH₃-NMe), 3.53 (t, 2 H, *J* = 6.8 Hz, H-14), 3.21 (s, 3 H, CH₃-NMe), 2.62 (dm, 2 H, *J* = 123.6 Hz, H-4), 1.76 (p, 2 H, *J* = 7.6 Hz, H-13), 1.67-1.20 (m, 16 H, H-5 to H-12). ¹³C NMR (101 MHz, CDCl₃) δ 167.7 (d, *J* = 67.7 Hz, C-1), 147.9 (dd, *J* = 67.5, 34.4 Hz, C-3), 118.0 (t, *J* = 67.2 Hz, C-2), 61.5 (CH₃-OMe), 45.8 (C-14), 32.7 (C-13), 32.2 (CH₃-NMe), 29.7-28.7 (m, 8x CH₂ C-4 to C-13), 27.0 (C-12). IR (neat); 2922, 2853, 1614, 1462, 1331, 1177, 1086, 997 cm⁻¹. HRMS calculated for [C₁₁¹³C₅H₃₀NO₂Cl + H]⁺: 309.1965, found 309.1966.

[1,2,3,4,5-¹³C₅]-14-Chloro-*N*-methoxy-*N*-methyl-tetradecanamide (30).



[1,2,3,4,5-¹³C₅]-(*E/Z*)-12-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide **29** (5.18 g, 16.8 mmol) was dissolved in EtOAc (200 mL). The solution was purged with argon under stirring and a catalytic amount of palladium 10% on charcoal was added. The reaction was stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere overnight. The palladium was removed by filtration over a plug of Celite and the rinsed with EtOAc followed by removal of the solvent under reduced pressure giving the desired product, a colorless oil (5.00 g, 16.1 mmol, 95%). *R_f* = 0.15 (10% EtOAc in petroleum ether). ¹H NMR (400 MHz, CDCl₃) δ 3.68 (s, 3 H, CH₃-OMe), 3.53 (t, 2 H, *J* = 6.4 Hz, H-14), 3.18 (s, 3 H, CH₃-NMe), 2.40 (dm, 2 H, *J* = 128.0 Hz, H-2), 1.81-1.73 (m 2 H, H-13), 1.60 (dm, 2 H, *J* = 130.4 Hz, H-3), 1.55-1.05 (m, H 18, H-4 to H-12). ¹³C NMR (101 MHz, CDCl₃) δ 174.9 (d, *J* = 53.5 Hz, C-1), 61.3 (CH₃-OMe), 45.3 (C-14), 32.8-31.6 (m, C-2, C-13 and CH₃-NMe), 30.0-29.0 (m, 7x CH₂ C-3 to C-12), 27.0 (CH₂ C-3 to C-12), 25.4-24.1 (m, 2x CH₂ C-3 to C-12). IR (neat) 2922, 2852, 1624, 1462, 1369, 1175, 997 cm⁻¹. HRMS calculated for [C₁₁¹³C₅H₃₂NO₂Cl + H]⁺: 311.2121, found 311.2123.

[2,3,4,5,6-¹³C₅]-15-Chloro-pentadec-1-ene (31).



[1,2,3,4,5-¹³C₅]-14-Chloro-*N*-methoxy-*N*-methyl-tetradecanamide **30** (3.76 g, 12.11 mmol) was dissolved in dry THF (50 mL) and was cooled to -78°C under protected atmosphere. DIBAL-H (1.5 M in toluene, 9.7 mL, 14.5 mmol, 1.2 eq) was added and the mixture was stirred for 1 hour at -78°C. The mixture was quenched with 1 M HCl aq (50 mL) at -78°C and was vigorously stirred to room temperature. The mixture was diluted with ether and was washed 2x with water and 1 x with brine. The water layers were extracted with ether and the combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo* giving crude [1,2,3,4,5 - ¹³C₅]-14-Chloro-tetradecanal, which was used without further purification. Methyltriphenylphosphonium bromide (8.65 g, 24.2 mmol, 2 eq) was suspended in dry THF (120 mL) and NaH (60% in mineral oil, 0.74 mg, 18.2 mmol, 1.5 eq) was added. The reaction mixture was refluxed for 3 h to form the ylide. (mixture turned to yellow) Then the crude [1,2,3,4,5-¹³C₅]-14-Chloro-tetradecanal dissolved in THF (12 mL) was then added to the ylide at 0 °C. The reaction mixture was stirred at room temperature overnight. The reaction was diluted with Et₂O and then washed 2x with water and 1x with brine. The water layers were extracted with Et₂O and the organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The product was purified by column chromatography (hexane) giving a colorless oil (2.48 g, 9.9 mmol, 82%). *R_f* = 0.95 (pentane). ¹H NMR (400 MHz, CDCl₃) δ 5.81 (dm, 1 H, *J* = 150.4 Hz),

5.01 (ddd, 1 H, $J = 17.7$ 6.4, 1.6 Hz, H-1_z), 4.92 (t, 1 H, $J = 10.8$ Hz, H-1_E), 3.53 (t, 2 H, $J = 6.4$ Hz, H-15), 2.03 (dm, $J = 126.4$ Hz, H-3), 1.76 (p, 2 H, $J = 7.0$ Hz, H-14), 1.56-1.05 (m, 20 H, H-4 to H-13). ^{13}C NMR (101 MHz, CDCl_3) δ 139.4 (d, $J = 42.4$ Hz, C-2), 114.5 (d, $J = 69.2$ Hz, C-1), 45.4 (C-15), 34.0 (m, C-3), 32.8 (C-14), 30.2-28.0 (m, 9x CH_2 C-4 to C-13), 26.9 (C-13). IR (neat); 2920, 2851, 1612, 1464, 990, 908 cm^{-1} .

[5,6,7,8,9- $^{13}\text{C}_5$]-[E]-18-Chloro-*N*-(*tert*-butyloxycarbonyl)-D-erythro-sphingosine (32). (2*S*,3*R*)-2-amino-*N*-(*tert*-butyloxycarbonyl)-1,3-dihydroxy-4-pentene (1.5 g, 6.9 mmol, 3 eq) and [2,3,4,5,6- $^{13}\text{C}_5$]-15-chloro-pentadec-1-ene **31** (0.57 g, 2.3 mmol) were dissolved in dry DCM (5 mL) and AcOH (26 μL , 0.46 mmol, 0.2 eq). The mixture was flushed with argon before addition of 2nd generation Grubbs catalyst (58 mg, 0.069 mmol, 0.03 eq). The reaction was refluxed for 2 days. The reaction mixture was concentrated *in vacuo* and purified by column chromatography (20% EtOAc in petroleum ether) giving pale brown viscous oil (882 mg, 2.01 mmol, 86%). $R_f = 0.44$ (1:1 EtOAc: petroleum ether). Mp 59-60°C. $[\alpha]_D^{20} : +8.6$ (C = 1.0, CHCl_3). ^1H NMR (400 MHz, CDCl_3) δ 5.80 (dm, $J = 148.0$ Hz, H-5), 5.55 (m, 1 H, H-4), 4.28 (m, 1 H, H-3), 3.80 (ddd, 2 H, $J = 92.8$, 11.6, 3.6 Hz, H-1), 3.59 (bs, 1 H, H-2), 3.53 (t, 2 H, $J = 6.8$ Hz, H-18), 3.30-3.00 (m, 2 H, 2x OH), 2.04 (dm, 2 H, $J = 125.2$ Hz, H-6), 1.80-1.72 (m, 2 H, H-17), 1.58-1.05 (m, 27 H, 3x CH_3 -*tBu* and H-7 to H-16). ^{13}C NMR (101 MHz, CDCl_3) δ 156.2 (C=O Boc), 134.1 (d, $J = 43.4$, C-5), 125.1 (d, $J = 71.7$ Hz, H-4), 74.8 (C-3), 62.8 (C-1), 55.6 (C-2), 45.2 (C-18), 32.9-31.7 (m, C-6 and C-17), 29.9-28.4 (m, 10x CH_2 C-7 to C-16 and 3x CH_3 -*tBu*), 27.0 (CH_2 C-7 to C-16), 14.1 (C_q -*tBu*). IR (neat) 3390, 2920, 2851, 1688, 1506, 1365 1169, 1053, cm^{-1} . HRMS calculated for $[\text{C}_{18}^{13}\text{C}_5\text{H}_{44}\text{NO}_4\text{Cl} + \text{H}]^+$: 439.2958, found 439.2970.

[5,6,7,8,9- $^{13}\text{C}_5$]-18-Azido-*N*-(*tert*-butyloxycarbonyl)-sphinganine (33). Chloro-D-erythro-sphingosine **32** (0.8 g, 1.82 mmol, 1.0 eq) was dissolved in EtOAc (10 mL). The solution was purged with argon under stirring and a catalytic amount of (PtO_2) was added. The reaction was stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere overnight. The palladium was removed by filtration over a plug of Celite and then rinsed with EtOAc followed by concentration *in vacuo*. The product was purified by column chromatography (30% EtOAc in petroleum ether) giving a white solid (0.44 g, 1.0 mmol, 86%). $R_f = 0.44$ (1:1 EtOAc: petroleum ether). Mp 74-75°C. $[\alpha]_D^{20} : +7.4$ (C = 1.0, CHCl_3). ^1H NMR (400 MHz, CDCl_3) δ 5.38 (m, 1 H, NH), 3.84 (ddd, 2 H, $J = 97.2$, 11.2, 2.8 Hz, H-1), 3.85-3.60 (m, 1 H, H-3), 3.50-3.46 (t, 3 H, $J = 6.8$ Hz, 1 H of H-2 and 2 H of H-18), 2.50 (bs, 1 H, OH), 2.40 (bs, 1 H, OH), 1.74-1.68 (m, 2 H, H-17), 1.60-1.05 (m, 35 H, 3x CH_3 -*tBu* and 13x CH_2 H-4 to H-16). ^{13}C NMR (101 MHz, CDCl_3) δ 156.2 (Boc), 74.6 (C-3), 62.8 (C-1), 54.9 (C-2), 45.3 (C-18), 34.6 (C-4), 32.8 (C-17), 29.9-25.7 (12x CH_2 C-5 to C-16 and 3x CH_3 -*tBu*), 14.2 (C_q -*tBu*). IR (neat); 3341, 2913, 2847, 1684, 1530, 1171 cm^{-1} . HRMS calculated for $[\text{C}_{18}^{13}\text{C}_5\text{H}_{46}\text{NO}_4\text{Cl} + \text{H}]^+$: 441.3115, found 441.3119.

Chloro-*N*-(*tert*-butyloxycarbonyl)-sphinganine (0.4 g, 0.9 mmol, 1.0 eq) was dissolved in dry DMF (4 mL). To the solution was added NaN_3 (0.16 g, 2.7 mmol, 3 eq) and a catalytic amount of NaI and the mixture was heated to 55 °C overnight. The mixture was diluted with ether and 2x washed with water and 1x with brine. The water layers were extracted with ether and the organic layers were combined, dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by column chromatography (30% EtOAc in petroleum ether) giving a white solid (0.44 mg, 0.9 mmol, 99%). $R_f = 0.40$ (1:1 EtOAc: petroleum ether). Mp 62-63 °C. $[\alpha]_D^{20} : +8.8$ (C = 1.0, CHCl_3). ^1H NMR (400 MHz, CDCl_3) δ 5.38 (m, 1 H, NH), 3.87 (ddd, 2 H, $J = 94.8$, 11.6, 3.6 Hz, H-1), 3.85-3.60 (m, 1 H, H-2), 3.55-3.48 (m, 1 H, H-3), 3.25 (t, 2 H, $J = 7.2$ Hz, H-18), 2.75 (bs, 1 H, OH), 2.65 (m, 1 H, OH), 1.80-1.00 (m, 37 H, 3x CH_3 -*tBu* and 13x CH_2 H-4 to H-17). ^{13}C NMR (101 MHz, CDCl_3) δ 156.2 (C=O), 74.6 (C-3), 62.8 (C-1), 54.9 (C-2), 51.6 (C-18), 34.4 (C-4), 29.9-25.7 (12x CH_2 C-5 to C-16 and 3x CH_3 -*tBu*), 14.2 (C_q -*tBu*). IR (neat) 3341, 2914, 2849, 2097, 1684, 1526, 1169, 906, 729 cm^{-1} . HRMS calculated for $[\text{C}_{18}^{13}\text{C}_5\text{H}_{46}\text{N}_4\text{O}_4 + \text{H}]^+$: 448.3519, found 448.3514.

[5,6,7,8,9-¹³C₅] - 18-Azido-sphinganine (22b). To azido-*N*-(tert-butyloxycarbonyl)-sphinganine (0.36 g, 0.8 mmol, 1 eq) was added a mixture of TFA:water (3:1, 8 mL) at 0 °C and stirred for 30 minutes. The mixture was diluted with toluene and concentrated *in vacuo*. The product was purified by column chromatography (9:1 CHCl₃:MeOH to 70:27:3 CHCl₃:MeOH:H₂O) giving a white TFA-solid (197 mg, 0.56 mmol, 71%). *R*_f = 0.34 (CHCl₃:MeOH:H₂O 70:27:3). $[\alpha]_D^{20}$ = -3.4 (C = 1.0, MeOH). Mp 82-83 °C. ¹H NMR (400 MHz, MeOD); 3.83 (dd, 1 H, *J* = 11.5, 4.0 Hz, H-1_a), 3.78 (m, 1 H, H-3), 3.70 (dd, 1 H, *J* = 11.5, 8.8 Hz, H-1), 3.27 (t, 2 H, *J* = 6.8 Hz, H-18), 3.19 (dt, 1 H, *J* = 8.4, 4.0 Hz, H-2), 1.58 (p, 2 H, *J* = 7.2 Hz, H-17), 1.53-1.05 (m, 26 H, H-4 to H-5). ¹³C NMR (101 MHz, MeOD); 70.34 (C-3), 58.97 (C-1), 58.37 (d, *J* = 3.0 Hz, C-2), 52.45 (C-18), 34.16 (d, *J* = 34.3 Hz, C-4), 30.8-30.0, 29.9, 27.8, 27.4-26.7 (13x CH₂ C-5 to C-17). IR (neat); 3340, 2916, 2849, 2099, 1645, 1215, 1161, 1049, 725 cm⁻¹. HRMS calculated for [C₁₃¹³C₅H₃₈N₄O₂ + H]⁺: 348.2994, found 348.2785.

7.2 References and notes.

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List of Publications

Gaucher disease and Fabry disease: new markers and insight in pathophysiology for two distinct glycosphingolipidoses

M. J. Ferraz, W. W. Kallemeijn, M. Mirzaian, D. H. Moro, A. R. A. Marques, P. Wisse, R. G. Boot, L. I. Willems, H. S. Overkleeft, J. M. Aerts, *Biochimica et Biophysica Acta-Molecular and Cells Biology of Lipids* **2014**, 1841 (5), 811-825.

Near-infrared labeled, ovalbumin loaded polymeric nanoparticles based on a hydrophilic polyester as model vaccine: In vivo tracking and evaluation of antigen-specific CD8⁺ T cell immune response

S. Rahimian, J. W. Kleinovink, M. F. Fransen, L. Mezzanotte, H. Gold, P. Wisse, H. S. Overkleeft, M. Amidi, W. Jiskoot, C. W. Lowik, F. Ossendorp, W. E. Hennink *Biomaterials* **2015**, 37, 469-477.

Mass spectrometric quantification of glucosylsphingosine in plasma and urine of type 1 Gaucher patients using an isotope standard

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Synthesis of a panel of carbon-13-labelled (glyco)sphingolipids

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Synthesis of 6-hydroxysphingosine and alpha-Hydroxy ceramide using a cross-metathesis strategy

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Lyso-glycosphingolipid abnormalities in different murine models of lysosomal storage disorders

M. J. Ferraz, A. R. A. Marques, P. Gaspar, M. Mirzaian, C. P. A. A. van Roomen, R. Ottenhoff, P. Alfonso, P. Irun, P. Giraldo, P. Wisse, C. S. Miranda, H. S. Overkleeft, J. M. Aerts, *Molecular Genetics and Metabolism* **2016**, *117* (2), 186-193.

Glycosylated cholesterol in mammalian cells and tissues: formation and degradation by multiple cellular beta-glucosidases

A. R. A. Marques, M. Mirzaian, H. Akiyama, P. Wisse, M. J. Ferraz, P. Gaspar, K. Ghauaurali-van der Vlugt, R. Meijer, P. Giraldo, P. Alfonso, P. Irun, M. Dahl, S. Karlsson, E. V. Pavlova, T. M. Cox, S. Scheij, M. Verhoek, R. Ottenhoff, C. P. A. A. van Roomen, N. S. Pannu, M. van Eijk, N. Dekker, R. G. Boot, H. S. Overkleeft, E. Blommaart, Y. Hirabayashi, J. M. Aerts, *Journal of Lipid Research* **2016**, *57* (3), 451-463.

Lysosomal glycosphingolipid catabolism by acid ceramidase: formation of glycosphingoid bases during deficiency of glycosidases

M. J. Ferraz, A. R. A. Marques, M. D. Appelman, M. Verhoek, A. Strijland, M. Mirzaian, S. Scheij, C. M. Quairy, D. Lahav, P. Wisse, H. S. Overkleeft, R. G. Boot, J. M. Aerts, *FEBS Letters* **2016**, *6*, 716-725.

Biochemical response to substrate reduction therapy versus enzyme replacement therapy in Gaucher disease type 1 patients

B. E. Smid, M. J. Ferraz, M. Verhoek, M. Mirzaian, P. Wisse, H. S. Overkleeft, C. E. Hollak, J. M. Aerts, *Orphanet Journal of Rare Diseases* **2016**, *11*, 28

Accurate quantification of sphingosine-1-phosphate in normal and Fabry disease plasma, cells and tissue by LC-MS/MS with C-13-encoded natural S1P as internal standard

M. Mirzaian, P. Wisse, M. J. Ferraz, A. R. A. Marques, T. L. Gabriel, C. P. A. A. van Roomen, R. Ottenhoff, M. van Eijk, J. D. C. Codée, G. A. van der Marel, H. S. Overkleeft, J. M. Aerts, *Clinica Chimica Acta* **2016**, *459*, 36-44.

Simultaneous quantitation of sphingoid bases by UPLC-ESI-MS/MS with identical ¹³C-encoded internal standards

M. Mirzaian, P. Wisse, M. J. Ferraz, A. R. A. Marques, P. Gaspar, S. V. Oussoren, K. Kytidou, J. D. C. Codée, G. A. van der Marel, H. S. Overkleeft, J. M. F. G. Aerts, *Clinical Chimica Acta* **2017**, *466*, 178-184.

Nederlandse Samenvatting.

Sphingolipiden zijn samen met sterolen en glycerophospholipiden hoofdbestanddelen van celmembranen. Deze lipiden zijn ondersteunende structurelementen, die niet alleen belangrijk zijn voor het functioneren van het membraan als barrière voor het transport van moleculen, maar bijvoorbeeld ook voor cel-cel herkenning en intercellulaire communicatie. Sphingolipiden bestaan uit een apolair deel, ceramide genaamd en een polair deel, dat zich buiten de cel bevindt. Het apolaire ceramide is weer opgebouwd uit sphingosine en een daaraan gekoppeld vetzuur. Het polaire gedeelte van sphingolipiden bestaat uit verschillende koolhydraten en fosfaten esters, die belangrijk zijn voor de verschillende functies van sphingolipiden.

In menselijke cellen zijn verschillende enzymen betrokken bij de opbouw en afbraak (het metabolisme) van sphingolipiden. Verstoring van het sphingolipide metabolisme door een defect enzym of zelfs het ontbreken van een betrokken enzym kan verschillende medische aandoeningen tot gevolg hebben. Om het inzicht in het sphingolipide metabolisme en de daaraan gekoppelde medische aandoeningen te vergroten is het beschikbaar hebben van gedefinieerde sphingolipiden alsmede analoga of derivaten daarvan van groot belang. Dergelijke verbindingen kunnen toegankelijk worden gemaakt door organische synthese. In dit kader beschrijft dit proefschrift het ontwerp en de synthese van verschillende gemodificeerde sphingolipiden.

Hoofdstuk 1 geeft een overzicht van verschillende synthetische gemodificeerde sphingolipiden, die gebruikt worden om het metabolisme van sphingolipiden te bestuderen. Het soort modificatie in sphingolipiden en de bijbehorende synthetische strategieën zijn daarbij als leidraad gebruikt.

Hoofdstuk 2 beschrijft de synthese van een kleine bibliotheek van verschillende koolstof-13-verrijkte (glyco)sphingolipiden. Deze koolstof-13 verrijkte sphingolipiden worden gebruikt als interne standaard voor bepalen en vergelijken van de concentratie van sphingolipiden in het plasma en urine van verschillende individuen. Uitgezonderd het molecuulgewicht, zijn de fysische-chemische eigenschappen van koolstof-13-verrijkte sphingolipiden identiek aan de overeenkomstige natuurlijk voorkomende sphingolipiden. Hierdoor zijn deze verbindingen ideaal om de concentratie van sphingolipiden in plasma en urine te bepalen en kunnen de verkregen waarden van gezonde individuen vergeleken worden met die van patiënten met een lysosomale sphingolipide stapelingsziekte. Tevens kan met deze bepalingen het ziekteverloop van patiënten gevolgd worden, waardoor de behandelingsmethode per patiënt geoptimaliseerd kan worden. Tenslotte kunnen de gesynthetiseerde koolstof-13-gelabelde sphingolipiden worden gebruikt voor het vinden van alternatieve metabole routes. De in dit hoofdstuk beschreven synthese van koolstof-

13-gelabeld sphingolipiden maakt gebruik een nieuwe synthetische strategie met $^{13}\text{C}_2$ -azijnzuur als uitgangsstof.

Hoofdstuk 3 is een vervolg op hoofdstuk 2 en is gericht op het optimaliseren van de koppeling van suikermoleculen aan beschermde sphingosine bouwstenen. Voor deze zogenoemde glycosyleringsreactie werd in hoofdstuk 2 een methode beschreven met de Boc-groep voor de bescherming van de amino functie in de sphingosine bouwsteen. Echter de Boc-groep bleek onvoldoende stabiel onder de zure condities van verschillende glycosyleringsreacties. Hierdoor waren de opbrengsten van sommige suikerkoppelingen laag terwijl glycosyleringen met andere suikermoleculen en *N*-Boc beschermde sphingolipiden zelfs onmogelijk waren. In hoofdstuk 3 wordt aangetoond dat het vervangen van de *N*-Boc door de baselabele *N*-Fmoc beschermende groep in sphingolipiden bouwstenen een uitstekende manier is om glycosyleringen met goede opbrengsten te bewerkstelligen.

Hoofdstuk 4 behandelt de synthese van fosfaat monoesters van koolstof-13-gelabelde sphingolipiden. Evenals de koolstof-13-gelabelde (glyco)sphingolipiden van hoofdstuk 2 kunnen deze verbindingen worden gebruikt als interne standaard bij de analyse van patiënten met een lysosomale stapelingsziekte. Ook hier werd gebruik gemaakt van het met koolstof-13-gelabeld *N*-Boc beschermde sphingosine. Voor de synthese van de doel fosfaat monoesters werden twee fosfor amidaat reagentia, geschikt voor de introductie van fosfaat monoesters ontwikkeld. Ook wordt aandacht besteed aan een eenvoudige zuiveringsmethode voor het moeilijk op te lossen sphingosine-1-fosfaat. Daarnaast wordt een nieuwe methode voor de *N*-acylering van gefosforyleerd sphingosine besproken.

Het onderwerp van **Hoofdstuk 5** is de ontwikkeling van nieuwe synthesesroutes voor zowel 6-hydroxyl-sphingosine als voor een alpha-hydroxy-vetzuur met behulp van een cross-metathese strategie. Het hoofdzakelijk in de huid voorkomende 6-hydroxy-sphingosine is het meest recente ontdekte sphingosine in de mens. De enige gepubliceerde synthetische strategie voor 6-hydroxyl-sphingosines maakt gebruik van een nucleophilic aanval van een beschermd alkyn op Garner aldehyde. Deze sterk basische condities beperken de modificatie mogelijkheden van 6-hydroxylsphingosine, die met een strategie, zoals die is beschreven in hoofdstuk 5 wordt omzeild. Door gebruik te maken van kruis-metathese werd een robuuste, milde en eenvoudige syntheseroute naar 6-hydroxy-sphingosine alsmede een bekende modificatie (zie hoofdstuk 1) mogelijk. Alpha-hydroxy ceramides zijn voorzien van een *N*-acyl alpha-hydroxy vetzuur. Ook dit vetzuur kon gesynthetiseerd worden met een vergelijkbare kruis-metathese strategie waarbij, een chiraal cyanohydrin als startmateriaal werd gebruikt.

Hoofdstuk 6 beschrijft het ontwerp en de synthese van gemodificeerde ceramides, die voorzien zijn van een aziridine functie. Twee aziridine-ceramide verbindingen werden

gesynthetiseerd vanuit azido-sphingosine. Deze aziridines kunnen worden getest als “activity-based probes” (ABPs) voor de glycosidase enzymen van het sphingolipide metabolisme en kunnen worden geëvalueerd naast de bekende ABPs als de van cyclophellitol afgeleide aziridines met eenzelfde configuratie als natuurlijk voorkomende suikers.

Curriculum Vitae

Nederlands

Patrick Wisse werd geboren op 20 april 1989 te Kapelle. In 2007 behaalde hij het VWO diploma met de profielen Natuur en Techniek en Natuur en Gezondheid aan het Goese Lyceum te Goes. Na de middelbare school volgde hij eerst de bacheloropleiding Molecular Science & Technology aan de Universiteit Leiden en de Technische Universiteit Delft, en vervolgens aan de Universiteit Leiden de masteropleiding Chemistry met als studierichting Design & Synthesis.

In 2010 voltooide hij zijn bachelor scriptie in de vakgroep Medicinal Chemistry van Prof. dr A. P. IJzerman, onder dagelijkse begeleiding van dr J. Brussee. In 2011-2012 werd de masterstage uitgevoerd in de groep Bio-Organic Synthesis van Prof. dr H. S. Overkleeft en Prof. dr G. A. van der Marel, onder dagelijkse begeleiding van dr H. Gold en dr G. Lutteke. De titel van zijn master thesis was 'The Synthesis of Tools to Study Sphinganine Metabolism'. In Oktober 2012 werd de masteropleiding succesvol afgesloten.

Onmiddellijk na het behalen van masterdiploma werd in Oktober 2012 begonnen met het hier beschreven promotieonderzoek onder begeleiding van Prof. dr H. S. Overkleeft en dr J. D. C. Codée. Delen van dit onderzoek zijn gepresenteerd in de vorm van posters op de NWO-CW sectie bijeenkomst Design and Synthesis in Lunteren (2013) en de Nederlandse chemie 'Chains' congressen in Veldhoven (2014, 2015). Daarnaast heeft hij een mondelinge presentatie gegeven op het KNCV Organische Chemie symposium in Wageningen (2015). Tijdens zijn promotietraject heeft hij deelgenomen aan het HRMS Summer school in Maastricht (2013).

Curriculum Vitea

English

Patrick Wisse was born in Kapelle on April 20 1989. In 2007, he finished his VWO education with the majors Life Science & Technology and Life Science & Health at the Goese Lyceum in Goes. After his secondary education he completed the bachelor study Molecular Science & Technology at Leiden University and Delft University of Technology and subsequently he completed the master study Chemistry with the research track Design & Synthesis at Leiden University.

In 2010, he did his bachelor internship in the group of Medicinal Chemistry of Prof. dr A. P. IJzerman, under supervision of dr J. Brussee. Between 2011-2012 he did his master internship in the Bio-Organic Synthesis group of Prof. dr H. S. Overkleeft and Prof. dr G. A. van der Marel, under the daily supervision of dr H. Gold and dr G. Lutteke. His master thesis was entitled 'The Synthesis of Tools to Study Sphinganine Metabolism'. In October 2012, he completed his master study.

In October 2012 he commenced the Ph.D. studies, that are described in this thesis, under supervision of Prof. dr H. S. Overkleeft and dr J. D. C. Codée. During his Ph.D. studies, parts of the research described in this thesis were presented on posters at the NWO-CW Synthetic and Design section meeting in Lunteren (2013) and the Chains conferences in Veldhoven (2014 and 2015). An oral presentation was given at the KNCV Organic Chemistry Symposium in Wageningen (2015). During his PhD. he participated in HRMS Summer school in Maastricht (2013).
