The synthesis of chemical tools for studying sphingolipid metabolism

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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 ($^{13}C_5$) 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 it's 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 deglucosylated 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.

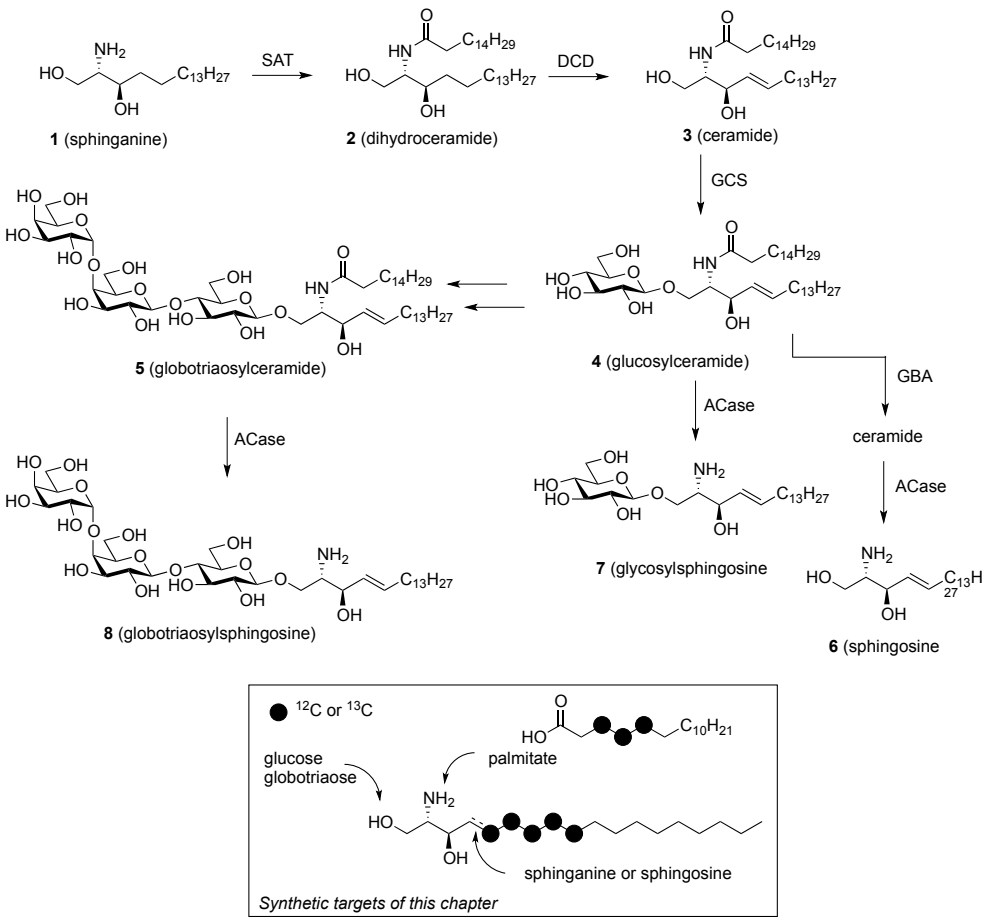


Figure 2.1 Partial overview of sphingolipid metabolism in man, and the target structures (insert) of the synthetic studies presented here. ACase: acid ceramidase; DCD: dihydroceramide dehydrogenase; GBA: glucocerebrosidase; GCS; glucosyceramide synthase; SAT: sphinganine acyl transferase.

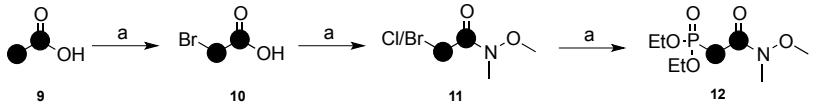
In contrast to common belief, it was found a few years ago that in tissue from Fabry patients, as well as in animal models, which are characterized by elevated levels of globotriaosylceramide due to genetically and partially disabled lysosomal α-galactosidase, the *N*-acyl chain of a portion of the accumulated 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 [$^{13}C_5$]-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 ^{13}C-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 [$^{13}C_5$]-sphingosine, the common backbone of all target structures, is crucial to the synthesis of the panel of [$^{13}C_n$]-sphingolipids. Based on literature precedence,[8,9] cross-metathesis of [$^{13}C_5$]-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 [^{13}C]-potassium cyanide and [$^{13}C_2$]-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 $^{13}C_2$-Horner-Wadsworth-Emmons reagent **12**.



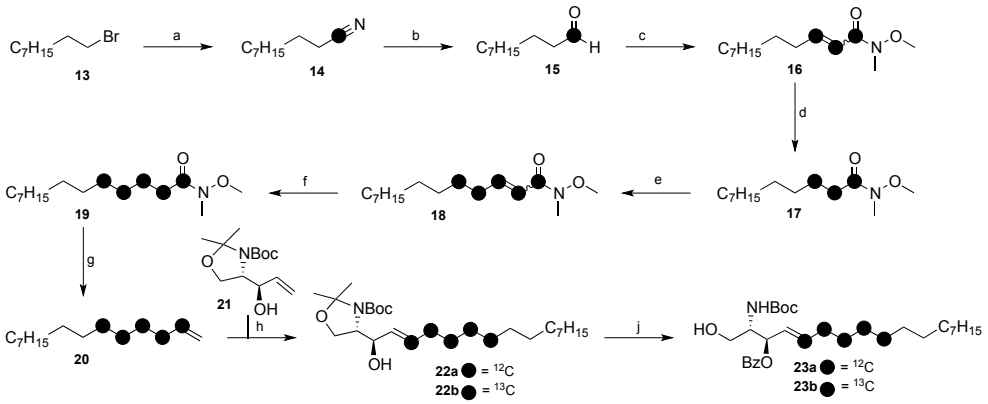
9 10 11 12

Reagents and conditions: (a) (i) TFAA (trifluoroacetic acid anhydride), Br_2, r.t., 20 h; (ii) water, 88 %; (b) (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, 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 [$^{13}C_3$]-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 $Ph_3P{=}CH_2$) into [$^{13}C_5$]-pentadeca-1-ene (**20**) in 93% yield.

With [$^{13}C_5$]-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*-butyloxycarbonyl) cleavage led to the isolation of the key building block **23**.

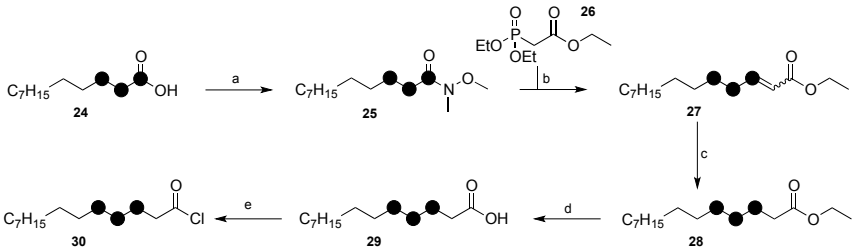
Scheme 2.2 Synthesis of the protected $^{13}C_5$-sphingosine **23**.



Reagents and conditions: (a) $K^{13}CN$, EtOH/H_2O, 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*-BuLi, THF, 0 °C, 10 min; (ii) [$^{13}C_1$]-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 [$^{13}C_3$]-dodecanal, which was added to a solution of (**12**, *n*-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*-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*-TsOH, r.t., 20 h, 63%.

[$^{13}C_3$]-Palmitoyl chloride **30** was obtained starting from commercially available [$^{13}C_3$]-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 [$^{13}C_3$]-palmitoyl chloride **30**.

Scheme 2.3 Synthesis of $^{13}C_3$-palmitoyl chloride **30**.



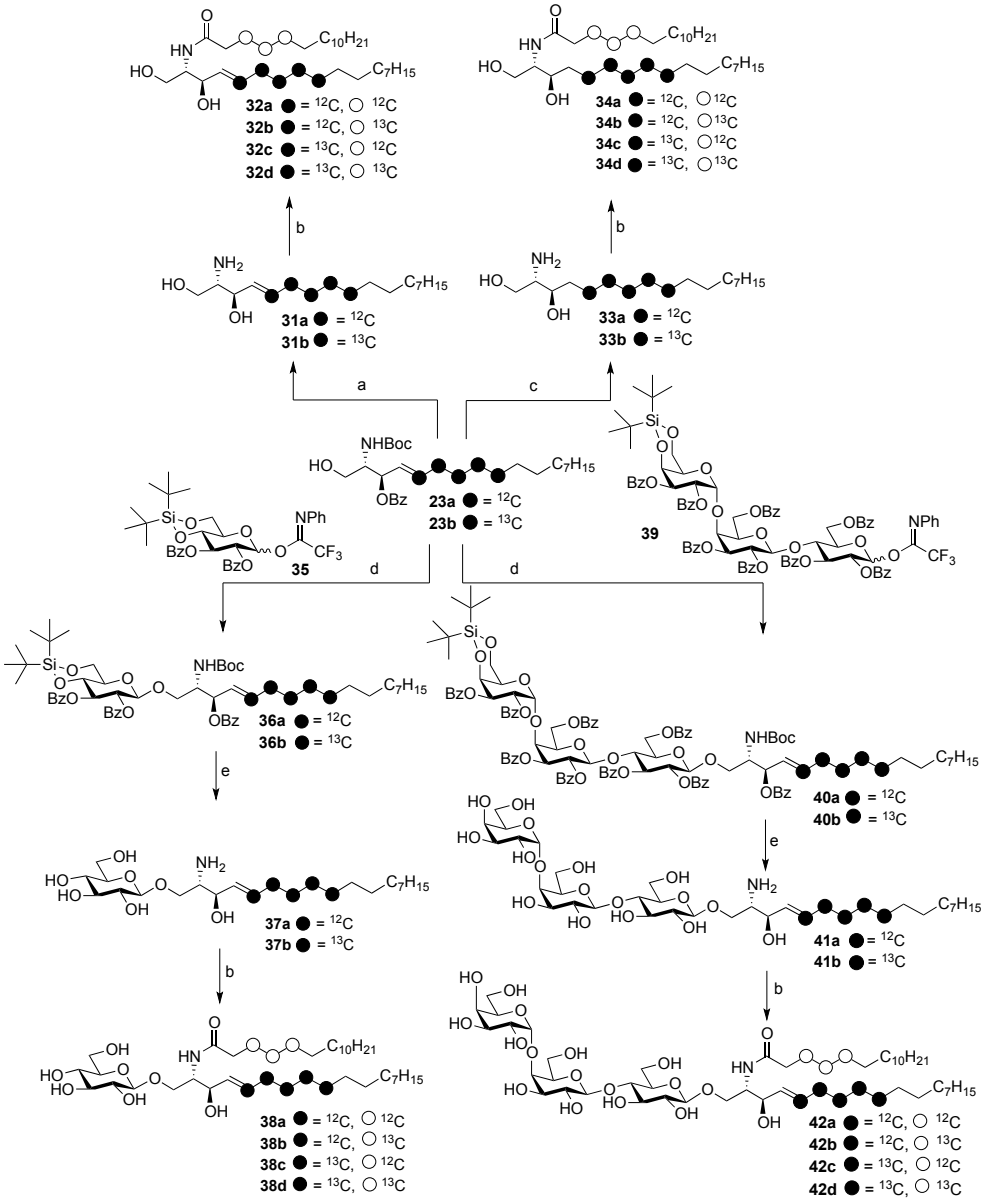
Reagents and conditions: (a) (i) oxalyl chloride, DMF, CH_2Cl_2, 0 °C to r.t., 2 h; (ii) *N,O*-dimethylhydroxylamine, -78 °C to r.t., 2 h, 98%; (b) DIBAL-H, THF, -78 °C, 30 min, to give crude [$^{13}C_3$]-tetradecanal, which was added to a solution of (**26**, *n*-BuLi, THF, 0 °C, 10 min), 0 °C to r.t., 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 [$^{13}C_5$]-sphingosine (**31b**; 59% yield). Both [$^{13}C_0$]-**31a** and [$^{13}C_5$]-**31b** were condensed with either [$^{13}C_0$]-palmitoyl chloride or [$^{13}C_3$]-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 dihydro-ceramides **34a**–**34d**.

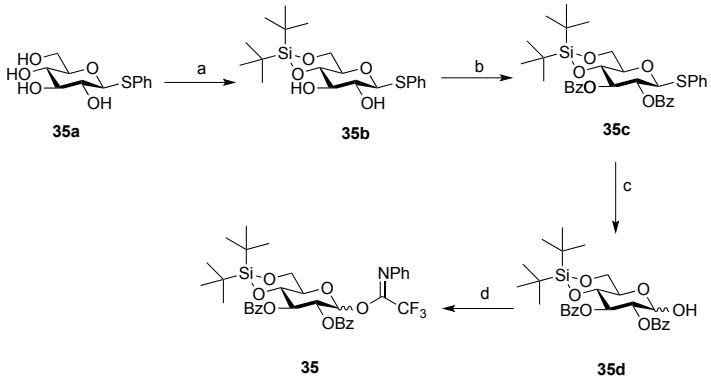
The glycosylated sphingolipids were assembled by reacting the labeled sphingosine alcohols with the appropriate glycosyl donors. Thus, *N*-phenyltrifluoroacetimidate glucose **35** (see Experimental Section for its synthesis; Scheme 2.5) and sphingosine **23a**/**b** were condensed in a reaction promoted 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 [$^{13}C_0$]-glucosylsphingosine (**37a**) and [$^{13}C_5$]-glucosylsphingosine (**37b**) were condensed with either [$^{13}C_0$]-palmitoyl chloride or [$^{13}C_3$]-palmitoyl chloride **30** to give the panel of labeled glucosylceramide derivatives **38a**–**38d**.

Scheme 2.4 Synthesis of panel of ^{13}C-labeled (glyco)sphingolipids.



Reagents and conditions: (a) (i) NaOMe, MeOH, r.t., 20 h; (ii) KOH, H_2O, r.t., 20 h; (iii) TFA, H_2O, 0 °C, 30 min, **31a**: 54%, **31b**: 59 %; (b) palmitoyl chloride, satd. aq. NaOAc, THF, r.t., 3 h; (c) (i) NaOMe, MeOH, r.t., 20 h; (ii) KOH, H_2O, r.t., 20 h; (iii) PtO_2, H_2 (g), EtOAc, r.t., 20 h; (iv) TFA, H_2O, 0 °C, 30 min, **33a**: 47%, **33b**: 52 %; (d) **35**/**39**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C, 1 h, **36a**: 49%, **36b**: 54%, **40a**: 60%, **44b**: 55%; (e) (i) HF/pyridine, THF/pyridine, r.t., 2 h; (ii) NaOMe, MeOH, r.t., 20 h; (iii) KOH, H_2O, r.t., 20 h; (iv) TFA, H_2O, 0 °C, 30 min, **37a**: 53%, **37b**: 49%, **41a**: 53%, **41b**: 48%.

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



Reagents and conditions: (a) tBu$_2$SiOTf$_2$, pyridine, DMF, –40 °C, 30 min, 77 %; (b) BzCl, pyridine, r.t., 3 h, 98%; (c) NIS, TFA, CH_2Cl_2, 0 °C, 3 h, 98%; (d) ClC(NPh)CF_3, $CsCO_3$, acetone, 0 °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 [$^{13}C_0$]-palmitoyl chloride or [$^{13}C_3$]-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 ^{1}H and ^{13}C NMR spectra. As a representative example, Figure 2 shows the ^{1}H and ^{13}C NMR spectra of $^{13}C_5$-globotriasylsphingosine **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 ^{1}H 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.

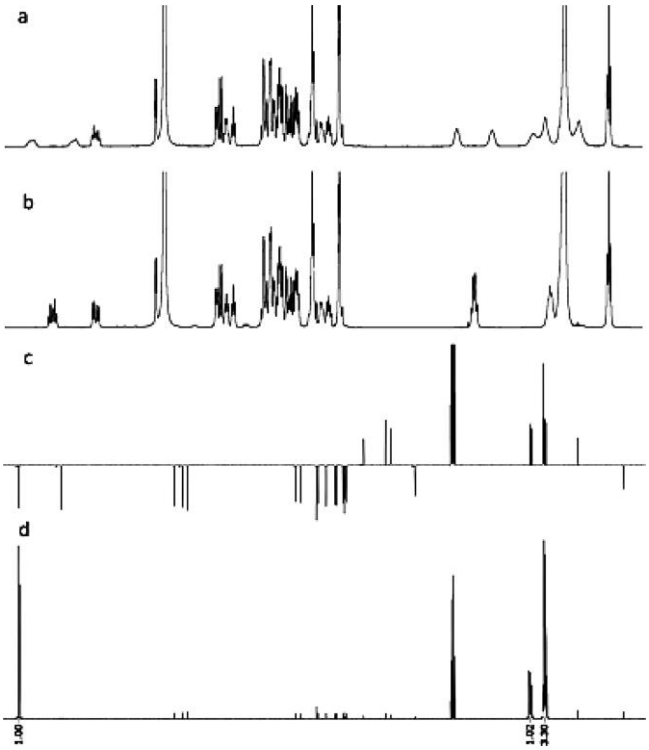


Figure 2.2 ^{1}H- and ^{13}C NMR spectra of globotriaosylsphingosine both in ^{13}C-enriched (**41b**) and unenriched (**41a**) form. (a) 400 MHz ^{1}H NMR spectrum ([D_4]methanol) of **41b**, in which the ^{13}C, ^{1}H coupling of the double-bond proton is apparent. (b) 400 MHz ^{13}C-decoupled ^{1}H NMR spectrum ([D_4]methanol) of **41b**. (c) 151.1 MHz ^{13}C NMR spectrum ([D_4]methanol) of **41a**. (d) 151.1 MHz ^{13}C NMR spectrum ([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.

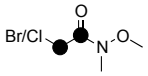
2.3 Experimental section

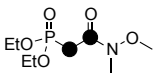
General Remarks: [$^{13}C_2$]-acetic acid (99.95% isotopically pure, product code CLM-105), potassium [^{13}C]-cyanide (99% isotopically pure, product code CLM-297), and [1,2,3-$^{13}C_3$]-myristic acid (99% isotopically pure, product code CLM-3665) were purchased from Cambridge Isotope Laboratories, 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 (MeOH/CH_2Cl_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 [$(NH_4)_6Mo_7O_{24}\cdot$ 4 H_2O (25 g/L), $(NH_4)_4Ce(SO_4)_6\cdot$ 2 H_2O (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, λ = 589 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 (δ = 0.00 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 □ 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 (Phe-nomenex, 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.

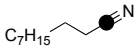
General producere for the synthesis of ceramides from the sphingosines. Sphingosine (0.1 mmol) was dissolved in THF (12 mL) and sat. aq. NaOAc (10 mL) was added. Palmitoyl chloride (0.13 mmol, 1.3 eq) was added and the reaction was stirred vigorously at room temperature for 3 hours. The mixture was diluted with THF (20 mL) and washed with water (10 mL). The water layer was extracted with THF (3x 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 C_4 column. Products were eluted using the following buffers: A: 25 nM NH_4OAc in MeOH/H_2O (3:1), B: acetonitrile (HPLC-grade). Purified products were lyophilised to remove water and traces of buffer salts. The symbol * in the NMR analysis stands for the palmitate group of the ceramide.

[$^{13}C_2$]-2-Bromoacetic acid (10). Trifluoroacetic anhydride (67.3 mL, 484 mmol, 3.0 eq) was slowly added to [1,2-$^{13}C_2$]-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 [$^{13}C_2$]-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-$^{13}C_2$]-2-Bromo-*N*-methoxy-*N*-methylacetamide and [1,2-$^{13}C_2$]-2-Chloro-*N*-methoxy-*N*-methylacetamide (11).  [$^{13}C_2$]-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-$^{13}C_2$]-2-Bromo-*N*-methoxy-*N*-methylacetamide and [1,2-$^{13}C_2$]-2-Chloro-*N*-methoxy-*N*-methylacetamide in a 4:1 ratio (as determined by ^{1}H- 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-$^{13}C_2$]-2-Bromo-*N*-methoxy-*N*-methyl-acetamide: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 (dd, 2 H, *J* = 154.0, 3.6 Hz, H-2), 3.80 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5 (d, *J* = 58.5 Hz, C=O), 61.6 ($CH_{3\text{-}OMe}$), 32.5 ($CH_{3\text{-}NMe}$), 25.1 (d, *J* = 58.4 Hz, CH_2); HRMS calculated for $[C_2{}^{13}C_2H_8NO_2Br + H]^+$: 183.9878, found 183.9877. [1,2-$^{13}C_2$]-2-Chloro-*N*-methoxy-*N*-methylacetamide: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.25 (dd, 2 H, J = 152.3, 4.4 Hz, H-2), 3.76 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5 (d, *J* = 57.2 Hz, C=O), 61.6 ($CH_{3\text{-}OMe}$), 40.7 (d, *J* = 57.7 Hz, CH_2), 32.5 ($CH_{3\text{-}NMe}$); HRMS calculated for $[C_2{}^{13}C_2H_8NO_2Cl + H]^+$: 140,0383 found 140.0381.

Diethyl-([1,2-$^{13}C_2$]-*N*-methoxy-*N*-methylcarbamoylmethyl) phosphonate (12).  [1,2-$^{13}C_2$]-2-Bromo/chloro-*N*-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); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.24 – 4.13 (m, 4 H, $CH_{2\text{-}OEt}$ x2), 3.79 (s, 3 H, $CH_{3\text{-}OMe}$), 3.22 (s, 3 H, $CH_{3\text{-}NMe}$), 3.16 (ddd, 2 H, *J* = 129.8, 21.9, 6.6 Hz, H-2), 1.35 (t, 6 H, *J* = 7.1 Hz, $CH_{3\text{-}OEt}$ 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 ($CH_{2\text{-}OEt}$ x 2), 60.9 ($CH_{3\text{-}OMe}$), 31.57 ($CH_{3\text{-}NMe}$), 30.9 (dd, *J* = 136.1, 53.1 Hz, H-2), 15.82, 15.76 ($CH_{3\text{-}OEt}$ x2); IR (neat): 2984, 1658, 1423, 1381, 1253, 1018, 961, 789 cm^{-1}; HRMS calculated for $[C_6{}^{13}C_2H_{18}NO_5P + H]^+$: 242.1063, found 242.1064.

[1-$^{13}C_1$]-Decanitrile (14).  [$^{13}C_1$]-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); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (dt, 2 H, *J* = 9.6, 7.1 Hz, H-2), 1.65 (m, 2 H, H-3), 1.44, (m, 2 H, H-4), 1.35 – 1.22 (m, 10 H, H-5 to H-9), 0.88 (t, 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 $[C_9{}^{13}CH_{19}N + H]^+$: 155.2623, found 155.2624.

[1-$^{13}C_1$]-Decanal (15). [1-$^{13}C_1$]-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. $NaHCO_3$ (400 mL). The water layers were extracted with ether (2 x 400 mL) and the combined organics were dried ($MgSO_4$), filtered over Celite, and concentrated *in vacuo*. Purification by column chromatography (0–10% DCM in petroleum ether) produced the title compound as a clear oil (10.4 g, 66.1 mmol, 92%). R_f = 0.22 (20% DCM in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (dt, 1 H, *J* = 169.8, 1.9 Hz), 2.42 (dtd, 2 H, *J* = 7.4, 6.2, 1.8 Hz), 1.62 (m, 2 H), 1.36 – 1.23 (m, 12 H), 0.88 (t, 3H, *J* = 6.9 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.0 (C=O), 43.9 (d, *J* = 38.8, C-2), 31.8, 29.35, 29.32, 29.2 (CH_2 x4), 29.1 (d, *J* = 3.4 Hz, C-4), 22.6 (CH_2), 22.0 (d, *J* = 1.6 Hz, C-3), 14.0 (C-10); IR (neat): 2922, 2855, 1728, 1466, 719 cm^{-1}.

[1,2,3-$^{13}C_3$]-(E/Z)-*N*-Methoxy-*N*-methyl-dodec-2-en-amide (16). Diethyl-([1,2-$^{13}C_2$]-*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-$^{13}C_1$]-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 (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (0–15% EtOAc in petroleum ether), giving [1,2,3-$^{13}C_3$]-(E)-*N*-Methoxy-*N*-methyl-dodec-2-en-amide (7.52 g, 30.8 mmol, 79%) and [1,2,3-$^{13}C_3$]-(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**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (dm, 1 H, *J* = 153.8 Hz, H-3), 6.38 (ddd, 1 H, *J* = 160.8, 15.4, 4.1 Hz, H-2), 3.70 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$), 2.23 (m, 2 H, H-4), 1.46 (m, 2 H, H-5), 1.35 –1.23 (m, 12 H, H-6 to H-11), 0.88 (t, 3 H, *J* = 6.8 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1 (d, *J* = 67.1 Hz, C=O), 148.0 (d, *J* = 71.6 Hz, C-3), 118.5 (dd, *J* = 71.6, 67.1 Hz, C-2), 61.6 ($CH_{3\text{-}OMe}$), 32.5 (m, C-4), 32.3 (m, $CH_{3\text{-}NMe}$), 31.9, 29.5, 29.4, 29.3 (CH_2 x4), 29.2 (d, *J* = 3.6 Hz, C-6), 28.3 (m, C-5), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2926, 5856, 1622, 1584, 1462, 1368, 1175, 993 cm^{-1}; HRMS calculated for [$C_{11}{}^{13}C_3H_{27}NO_2H$]$^+$: 245.2215, found 245.2216; (Z-isomer, **16Z**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (dd, 1 H, *J* = 161.8, 11.5 Hz, H-2), 6.11 (dm, 1 H, *J* = 152.0 Hz, H-3), 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.21 (s, 3 H, $CH_{3\text{-}NMe}$), 2.61 (m, 2 H, H-4), 1.43 (m, 2 H, H-5), 1.35 – 1.22 (m, 12 H, H-6 to H-11), 0.88 (t, 3H, *J* = 6.9 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.6 (d, *J* = 63.6, C=O), 147.8 (d, J = 67.1 Hz, C-3), 117. 9 (dd, *J* = 67.1, 63.6 Hz, C-2), 61.5 ($CH_{3\text{-}OMe}$), 31.9, 31.6 ($CH_{3\text{-}NMe}$)‡, 29.6, 29.5 (CH_2 x3), 29.38 (d, J = 4.0 Hz, C-6), 29.35 – 29.29 (m, CH_2 x2), 29.1 (m, C-3), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2925, 2855, 1618, 1459, 1334, 1178, 996, 776 cm^{-1}; HRMS calculated for [$C_{11}{}^{13}C_3H_{27}NO_2$ + H]$^+$: 245.2215, found 245.2216.

[1,2,3-$^{13}C_3$]-*N*-methoxy-*N*-methyl-dodecanamide (17). [1,2,3-$^{13}C_3$]-(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-$^{13}C_3$]-*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); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.18 (s, 3 H, $CH_{3\text{-}NMe}$), 2.41 (dm, 2 H, *J* = 127.3 Hz, H-2), 1.62 (dm, 2 H, *J* = 127.9 Hz, H-3), 1.35 – 1.23 (m, 16 H, H-4 to H-11), 0.88 (t, 3 H, *J* = 6.8 Hz, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.6 (bd, *J* = 51.5 Hz, C=O), 61.1 ($CH_{3\text{-}OMe}$), 31.9 ($CH_{3\text{-}NMe}$), 31.8 (dd, *J* = 51.5, 37.5 Hz, C-2), 29.7 – 29.1 (m, CH_2 x7), 24.6

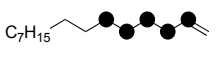
(dd, *J* = 34.9, 1.3 Hz, C-3) , 22.6 (CH_2), 14.1 (C-12); IR (neat): 2923, 2854, 1627, 1464, 1369, 1174, 1119, 998, 722, 436 cm^{-1}; HRMS calculated for $[C_{11}{}^{13}C_3H_{29}NO_2 + H]^+$: 247.2372, found 247.2373.

[1,2,3,4,5-$^{13}C_5$]-(E/Z)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (18). [1,2,3-$^{13}C_3$]-*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. $KHSO_4$ (100 mL) and diethylether (300 mL). The two-phase system was stirred vigorously for 30 min and was then dried with $MgSO_4$ followed by Na_2SO_4. The solids were filtered and washed with diethylether (200 mL). The eluate was concentrated *in vacuo*, giving crude [1,2,3-$^{13}C_3$]-dodecanal (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-$^{13}C_3$]-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 (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography (5–15% EtOAc in petroleum ether) giving [1,2,3,4,5-$^{13}C_5$]-(E)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide (3.05 g, 11.1 mmol, 70%) and [1,2,3,4,5-$^{13}C_5$]-(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**) ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.98 (dm, 1 H, *J* = 153.8 Hz, H-3), 6.39 (ddm, 1 H, J = 161.1, 15.4 Hz, H-2), 3.70 (s, 3 H, $CH_{3\text{-}OMe}$), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$), 2.23 (ddt, 2 H, *J* = 126.2, 7.0, 6.1 Hz, H-4), 1.60 – 1.20 (m, 18 H, H-5 to H-13), 0.88 (t, 3 H, *J* = 7.0 Hz, H-14); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.1 (dd, *J* = 67.1, 6.1 Hz, C=O), 148.0 (ddd, *J* = 71.6, 41.8, 2.1 Hz, C-3), 118.6 (dddd, *J* = 71.6, 67.1, 3.6, 1.5 Hz, C-2), 61.6 ($CH_{3\text{-}OMe}$), 32.5 (dddd, *J* = 41.8, 33.7, 6.1, 1.5 Hz, C-4), 32.3 ($CH_{3\text{-}NMe}$), 31.9 (CH_2), 29.6 – 29.0 (m, CH_2 x6), 28.3 (ddd, J = 33.7, 3.6, 2.1 Hz, C-5), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2924, 2854, 1618, 1583, 1464, 1368, 991 cm^{-1}; HRMS Calculated for $[C_{11}{}^{13}C_5H_{31}NO_2 + H]^+$: 275.2595, found 275.2595; (Z-isomer, **18Z**) ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.23 (dm, 1 H, *J* = 160.7 Hz, H-2), 6.12 (dm, 1 H, *J* = 152.0 Hz, H-3), 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.21 (s, 3 H, $CH_{3\text{-}NMe}$), 2.62 (dm, 2 H, *J* = 125.3 Hz, H-4), 1.59 – 1.20 (m, 18 H, H-5 to H-13), 0.88 (t, 3 H, *J* = 7.1 Hz, H-14); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.6 (dm, *J* = 67.1 Hz, C=O), 147.8 (dd, *J* = 69.9, 35.2 Hz, C-3), 117. 9 (dd, J = 69.9, 67.1 Hz, C-2), 61.4 ($CH_{3\text{-}OMe}$), 32.0 ($CH_{3\text{-}NMe}$)‡, 31.9 (CH_2), 30.2 – 28.4 (m, CH_2 x8), 22.7 (CH_2), 14.1 (C-12); IR (neat): 2923, 2854, 1618, 1464, 1331, 1176, 1086, 999, 775 cm^{-1}; HRMS calculated for $[C_{11}{}^{13}C_5H_{31}NO_2 + H]^+$: 275.2595, found 275.2595.

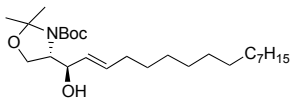
[1,2,3,4,5-$^{13}C_5$]-*N*-methoxy-*N*-methyl-tetradecanamide (19). [1,2,3,4,5-$^{13}C_5$]-(E/Z)-*N*-Methoxy-*N*-methyl-tetradec-2-enamide **18E/Z** (3.20 g, 11.66 mmol, 1.0 eq) was dissolved in EtOAc (100 mL). The solution was bubbled with argon under stirring, before addition of palladium (10% on charcoal) (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-$^{13}C_5$]-*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); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.18 (s, 3 H, $CH_{3\text{-}NMe}$), 2.41 (dm, 2 H, J = 128.4 Hz, H-2), 1.62 (dm, 2 H, *J* = 127.1 Hz, H-3), 1.46 – 1.12 (m, 20 H, H-4 to H-13), 0.88 (t, 3 H, *J* = 7.1 Hz, H-14); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.8 (dm, *J* = 51.5 Hz, C=O), 61.1 ($CH_{3\text{-}OMe}$), 32.1 ($CH_{3\text{-}NMe}$)‡, 31.9 (dd, *J* = 51.5, 35.6 Hz, C-2), 29.7 – 29.1 (m, CH_2 x9), 24.6 (m, C-3), 22.6 (CH_2), 14.1 (C-14).; IR (neat): 2922, 2853, 1628, 1458, 1370, 1175, 996, 721 cm^{-1}; HRMS calculated for $[C_{11}{}^{13}C_5H_{33}NO_2 + H]^+$: 277.2751, found 277.2752.

[2,3,4,5,6-$^{13}C_5$]-Pentadec-1-ene (20). [1,2,3,4,5-$^{13}C_5$]-*N*-Methoxy-*N*-methyl-tetradecanamide **19** (1.57 g, 5.72 mmol, 1.0 eq) was dissolved in anhydrous THF (55 mL) and $LiAlH_4$ (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. $KHSO_4$ (40 mL) and diethylether (100 mL). The resulting two phase mixture was stirred vigorously for 30 min and then dried with $MgSO_4$, and then Na_2SO_4. The solids were filtered and washed with diethylether (100 mL). The eluate was concentrated *in vacuo* giving crude [1,2,3,4,5-$^{13}C_5$]-tetradecanal (1.24 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-$^{13}C_5$]-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 (Na_2SO_4), 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); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (dm, 1 H, *J* = 150.3 Hz, H-2), 4.99 (dd, 1 H, *J* = 17.1, 6.5 Hz, H-1$_Z$), 4.92 (t, 1 H, *J* = 10.8 Hz, H-1$_E$), 2.03 (dm, 2 H, *J* = 125.4 Hz, H-3), 1.57 – 1.11 (m, 22 H, H-4 to H-14), 0.88 (t, 3 H, *J* = 6.8 Hz, H-15); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.2 (dm, *J* = 42.1 Hz, C-2), 114.0 (dd, *J* = 69.1, 3.1 Hz, C-1), 33.9 (m, C-3), 32.0 (CH_2), 29.9 – 28.6 (m, CH_2 x9), 22.7 (CH_2), 14.1 (C-15); IR (neat): 2922, 2853, 1628, 1458, 1370, 1175, 1117, 996, 721 cm^{-1}.

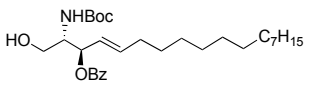
(E)-1,2-*O,N*-Isopropyliden-*N*-(*tert*-butoxycarbonyl)-D-*erythro*-sphingosine (22a). (2S,3R)-2-Amino-*N*-(*tert*-butyloxycarbonyl)-1,3-dihydroxy-1,2-*O,N*-isopropylidene-4-pentene **21** (1 g, 4.0 mmol, 1.0 eq) and pentadec-1-ene (1.70 g, 8.0 mmol, 2.0 eq) were dissolved in anhydrous DCM (4 mL) and 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); $[\alpha]_D^{22}$: −26 (c = 0.25 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6, 363 °K) δ 5.56 (dt, 1 H, *J* = 15.8, 6.5 Hz, H-5), 5.45 (ddd, 1 H, *J* =15.8, 6.6, 1.1 Hz, H-4), 4.61 (bs, 1 H, OH), 4.03 (m, 1 H, H-3), 3.93 (bd, 1 H, *J* = 8.5 Hz, H-1$_a$), 3.83 (bt, 1 H, *J* = 7.3 Hz, H-1$_b$), 3.75 (m, 1 H, H-2), 1.98 (m, 2 H, H-6)m 1.48 (s, 3 H, $CH_{3\text{-acetonide}}$), 1.43 (m, 12 H, $CH_{3\text{-acetonide}}$ and $CH_{3\text{-}t\text{Bu-Boc}}$), 1.39-1.20 (m, 22 H, H-7 to H-17), 0.87 (t, 3 H, *J* = 6.6 Hz, H-18); ^{13}C NMR (100 MHz, DMSO-d_6, 363 °K) δ 151.3 (C=O$_{Boc}$), 130.8 (C-5) 130.4 (C-4), 92.8 (C$_{q\text{-acetonide}}$), 78.7 (C$_{q\text{-Boc}}$), 71.4 (C-3), 63.7 (C-1), 61.0 (C-2), 31.2 (C-6), 30.8, 28.5 (x4), 28.4, 28.2, 28.12, 28.06, 27.7 (x3), 26.2, 21.5, (C-7 to C-17, $CH_{3\text{-tBu-Boc}}$ and $CH_{3\text{-acetonide}}$ x2), 13.2 (C-18). IR (neat): 3436, 2924, 2854, 1702, 1381, 1365, 1255, 1173, 1097, 848, 766 cm^{-1}; HRMS calculated for $[C_{26}H_{49}NO_4 + H]^+$: 440.3734, found 440.3733.



(E)-[5,6,7,8,9-$^{13}C_5$]-1,2-*O,N*-Isopropylidene-*N*-(*tert*-butoxycarbonyl)-D-*erythro*-sphingosine (22b) (2S,3R)-2-Amino-*N*-(*tert*-butyloxycarbonyl)-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-$^{13}C_5$]-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); $[\alpha]_D^{22}$: −19 (c = 0.5 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$, 363 °K) δ 5.55 (dm, 1 H, *J* = 152.0 Hz, H-5), 5.44 (m, 1 H, H-4), 4.60 (bd, 1 H, *J* = 5.4 Hz, OH), 4.05 (m, 1 H, H-3), 3.94 (dd, 1 H, *J* = 8.6, 2.0 Hz, H-

1_a), 3.82 (dd, 1 H, *J* = 8.6, 6.1 Hz, H-1_b), 3.75 (td, 1 H, *J* = 6.1, 2.0 Hz, H-2), 1.98 (dm, 2 H, J = 124.2 Hz, H-6), 1.56 – 1.06 (m, 37 H, $CH_{3\text{-}t\text{Bu-Boc}}$, $CH_{3\text{-acetonide}}$ and H-7 to H-17), 0.87 (t, 3 H, *J* = 6.9 Hz, H-18); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (dm, 1 H, *J* = 149.4 Hz, H-5), 5.45 (dd, 1 H, *J* = 15.4, 6.0 Hz, H-4), 4.39 – 3.74 (m, 5 H, H-3, H-2, H-1 and OH), 2.04 (dm, 2 H, *J* = 125.2 Hz, H-6), 1.72 – 1.01 (m, 37 H, $CH_{3\text{-}t\text{Bu-Boc}}$, $CH_{3\text{-acetonide}}$ and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{1}H NMR (400 MHz, $CDCl_3$, ^{13}C-decoupled) δ 5.74 (dt, 1 H, *J* = 15.4, 6.6 Hz, H-5), 5.45 (dd, 1 H, *J* = 15.4, 6.4 Hz, H-4), 4.39 – 3.74 (m, 5 H, H-3, H-2, H-1 and OH), 2.04 (q, 2 H, *J* = 7.0 Hz, H-6), 1.71 – 1.16 (m, 37 H, $CH_{3\text{-}t\text{Bu-Boc}}$, $CH_{3\text{-acetonide}}$ and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{13}C NMR (100 MHz, DMSO-d_6, 363 °K) δ 151.3 ($C{=}O_{Boc}$), 130.8 (d, *J* = 42.3 Hz, C-5), 130.4 (d, *J* = 73.4 Hz, C-4), 92.8 ($C_{q\text{-acetonide}}$), 78.4 ($C_{q\text{-Boc}}$), 71.4 (d, *J* = 5.2 Hz, C-3), 63.7 (C-1), 61.0 (d, *J* = 2.7 Hz, C-2), 31.9 – 30.5 (m, C-6_{Sp} and $CH_{2\text{-Sp}}$), 29.7 – 26.1 ($CH_{2\text{-Sp}}$ x10, $CH_{3\text{-}t\text{Bu-Boc}}$ and $CH_{3\text{-acetonide}}$ x2), 21.5 ($CH_{2\text{-Sp}}$), 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 $[C_{21}{}^{13}C_5H_{49}NO_4 + H]^+$: 445.3902, found 445.3902.

3-O-Benzoyl-*N*-(*tert*-butoxycarbonyl)-D-*erythro*-sphingosine (23a). (E)-1,2-*O,N*-Isopropyliden-*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. $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organic layers were dried (Na_2SO_4), 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*-butyloxycarbonyl)-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 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6, 363 °K) δ 8.00 (dm, 1 H, *J* = 7.9 Hz, H_{arom}), 7.63 (m, 1 H, H_{arom}), 7.55-7.47 (m, 2 H, H_{arom}), 5.82 (bs, 1 H, H-3), 5.75 (dt, 1 H, *J* = 15.4, 6.5 Hz, H-5), 5.53 (ddd, 1 H, *J* = 15.4, 6.2, 1.4 Hz, H-4), 4.09 (m, 1 H, H-2), 4.06-3.97 (m, 2 H, H-1_a and H-1_b), 2.01 (m, 2 H, H-6), 1.43 (s, 9 H, $CH_{3\text{-}t\text{Bu-Boc}}$), 1.40 (s, 3 H, $CH_{3\text{-acetonide}}$), 1.36-1.17 (m, 25 H, $CH_{3\text{-acetonide}}$ and H-7 to H-17), 0.86 (t, 3 H, H-18); ^{13}C NMR (100 MHz, DMSO-d_6, 363 °K) δ 164.5 ($C{=}O_{Bz}$), 134.4 (C-5), 132.7 (CH_{arom}), 129.7 ($C_{q\text{-arom}}$), 128.9, 128.1 (CH_{arom} x2), 125.4 (C-4), 93.2 ($C_{q\text{-acetonide}}$), 79.1 ($C_{q\text{-Boc}}$), 73.4 (C-3), 62.9 (C-1), 59.1 (C-2), 31.1, 30.8, 28.5 (x2), 28.4 (x2), 28.4, 28.2, 27.8 (x2), 27.6 (x2), 21.5 (C-7 to C-17, $CH_{3\text{-}t\text{Bu-Boc}}$ and $CH_{3\text{-acetonide}}$ x2), 13.3 (C-18); IR (neat): 2924, 2854, 1724, 1701, 1365, 1268, 1097, 1070, 855, 709 cm^{-1}; HRMS calculated for $[C_{33}H_{53}NO_5 + Na]^+$; 566.3816, found 566.3814.



1,2-*O,N*-Isopropylidene-3-*O*-benzoyl-*N*-(*tert*-butyloxycarbonyl)-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 $NaHCO_3$ (60 mL) and brine (50 mL). The water layers were back extracted with EtOAc (60 mL). The combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (10% EtOAc in petroleum ether) produced the title compound as a clear waxy solid (0.25 g, 0.50 mmol, 54%; 88% based on recovering starting material). R_f = 0.07 (10% EtOAc in petroleum ether); $[\alpha]_D{}^{22}$: +15 (c = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (dm, 2 H, *J* = 7.5 Hz, H_{arom}), 7.57 (t, 1 H, *J* = 7.4 Hz, H_{arom}), 7.44 (t, 2 H, *J* = 7.7 Hz, H_{arom}), 5.87 (dt, 1 H, *J* = 14.9, 6.6 Hz, H-5), 5.60 (dd, 1 H, *J* = 14.9, 7.7 Hz,H-4), 5.53 (t, 1 H, *J* = 7.3 Hz, H-3), 5.12 (d, 1 H, *J* = 8.9 Hz, NH_{Boc}), 3.95 (m, 1 H, H-2), 3.76-3.67 (m, 2 H, H-1_a and H-1_b). 2.82 (bs, 1 H, OH), 2.05 (m, 2 H, H-6), 1.43 (s, 9 H, $CH_{3\text{-}t\text{Bu-Boc}}$), 1.40-1.20 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 ($C{=}O_{Bz}$), 155.8 ($C{=}O_{Boc}$), 137.3 (C-5), 133.2 (CH_{arom}), 129.8 ($C_{q\text{-arom}}$), 129.7, 128.4 (CH_{arom} x2), 124.6 (C-4), 79.6 ($C_{q\text{-Boc}}$), 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 ($CH_{3\text{-}t\text{Bu-Boc}}$), 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 $[C_{30}H_{49}NO_5 + Na]^+$: 526.3503, found 526.3500.

[5,6,7,8,9-$^{13}C_5$]-3-*O*-Benzoyl-*N*-(*tert*-butoxycarbonyl)-D-*erythro*-sphingosine (23b). [5,6,7,8,9-$^{13}C_5$]-1,2-*O,N*-Isopropylidene-*N*-(*tert*-butyloxycarbonyl)-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. $NaHCO_3$ (50 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography (1.5% EtOAc in petroleum ether) giving [5,6,7,8,9-$^{13}C_5$]-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); $[\alpha]_D^{22}$: −30 (c = 0.5 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6, 363 °K) δ 8.00 (d, 2 H, *J* = 7.6 Hz, H_{arom}), 7.64 (t, 1 H, *J* = 7.4 Hz, H_{arom}), 7.52 (t, 2 H, *J* = 7.6 Hz, H_{arom}), 5.82 (bs, 1 H, H-3), 5.75 (dm, 1 H, *J* = 149.2 Hz, H-5), 5.53 (m, 1 H, H-4), 4.15 – 3.97 (m, 3 H, H-2, H-1_a and H-1_b), 2.04 (dm, 2 H, *J* = 126.1 Hz, H-6), 1.54 – 1.01 (m, 37 H, $CH_{3\text{-}tBu\text{-}Boc}$, $CH_{3\text{-}acetonide}$ x2 and H-7 to H-17), 0.86 (t, 3 H, *J* = 6.3 Hz, H-18); ^{13}C NMR (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 ($C_{q\text{-}arom}$), 128.9, 128.2 (CH_{arom} x2), 125.2 (d, J = 72.2 Hz, C-4), 93.2 ($C_{q\text{-}acetonide}$), 79.2 ($C_{q\text{-}Boc}$), 73.4 (d, *J* = 5.6 Hz, C-3), 62.9 (C-1), 59.1 (C-2), 31.8 – 30.4 (m, C-6 and CH_2), 28.8 – 27.3 (m, CH_2 x9, $CH_{3\text{-}tBu\text{-}Boc}$ and $CH_{3\text{-}acetonide}$ x2), 21.6 (CH_2), 13.4 (C-18); The same sample in $CDCl_3$ at room temperature shows two rotamers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 2 H, *J* = 7.4 Hz, H_{arom}), 7.55 (t, 1 H, *J* = 7.4 Hz, H_{arom}), 7.44 (t, 2 H, *J* = 7.6 Hz, H_{arom}), 5.93 – 5.82 (m, 1 H, H-3), 5.82 (dm, 1 H, *J* = 149.8 Hz, H-5), 5.46 (m, 1 H, H-4), 4.25 – 4.10 (m, 1.5 H, H-2, H-1_a), 4.07 – 3.96 (m, 1.5 H, H-2, H-1_b), 2.03 (dm, 2 H, *J* = 125.7 Hz, H-6), 1.58 – 1.00 (m, 37 H, $CH_{3\text{-}tBu\text{-}Boc}$, $CH_{3\text{-}acetonide}$ x2 and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.9 Hz, H-18); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 165.4 ($C{=}O_{Bz}$ x2), 152.5, 151.7 ($C{=}O_{Boc}$ x2), 135.8 (d, *J* = 42.6 Hz, C-5) 135.7 (d, *J* = 42.6 Hz, C-5), 132.9, 132.8 (CH_{arom} x2), 130.5, 130.3 ($C_{q\text{-}arom}$ x2), 129.8 (CH_{arom}), 128.3 (CH_{arom}), 125.0 (d, *J* = 72.8 Hz, C-4), 94.6, 94.0 ($C_{q\text{-}acetonide}$ x2), 80.4, 80.2 ($C_{q\text{-}Boc}$ x2), 74.4 (d, *J* = 5.6 Hz, C-3), 74.2 (d, *J* = 5.6 Hz, C-3), 63.70, 63.66 (C-1 x2), 60.00, 59.97 (C-2 x2), 32.8 – 31.7 (m, C-6 and CH_2), 29.8 – 28.2 (m, CH_2 x9, $CH_{3\text{-}tBu\text{-}Boc}$ and $CH_{3\text{-}acetonide}$ x2), 22.7 (CH_2), 14.1 (C-18); HRMS calculated for $[C_{28}{}^{13}C_5H_{53}NO_5 + Na]^+$: 571.3984, found 571.3982.

[5,6,7,8,9-$^{13}C_5$]-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. $NaHCO_3$:water 2:1 (60 mL) and brine (50 mL). The water layer was extracted with EtOAc (60 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography (5-10% EtOAc in petroleum ether) produced the title compound **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); $[\alpha]_D^{22}$: +16 (c = 0.5 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dm, 2 H, *J* = 7.8 Hz, H_{arom}), 7.57 (tt, 1 H, *J* = 7.0, 1.5 Hz, H_{arom}), 7.45 (t, 2 H, *J* = 7.8 Hz, H_{arom}), 5.88 (dm, 1 H, *J* = 149.8 Hz, H-5), 5.60 (m, 1 H, H-4), 5.52 (m, 1 H, H-3), 5.08 (d, 1 H, *J* = 8.9 Hz, NH_{Boc}), 3.93 (m, 1 H, H-2), 3.76 – 3.67 (m, 2 H, H-1_a and H-1_b), 2.66 (bs, 1 H, OH), 2.08 (dm, 2 H, *J* = 125.5 Hz, H-6), 1.58 – 1.01 (m, 31 H, $CH_{3\text{-}tBu\text{-}Boc}$ and H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3 ($C{=}O_{Bz}$), 155.8 ($C{=}O_{Boc}$), 137.4 (d, *J* = 42.5 Hz, C-5), 133.3 (CH_{arom}), 129.80 ($C_{q\text{-}arom}$), 129.75, 128.4 (CH_{arom} x2), 124.6 (d, *J* = 71.5 Hz, C-4), 79.7 ($C_{q\text{-}Boc}$), 74.9 (d, *J* = 5.4 Hz, C-3), 61.9 (C-1), 54.6 (C-2), 33.0 – 31.6 (C-6 and CH_2), 29.8 – 28.1 (CH_2 x9 and $CH_{3\text{-}tBu\text{-}Boc}$), 22.7 ($CH_2$), 14.1 (C-18); IR (neat): 3372, 2922, 2853, 1696, 1505, 1452, 1267, 1169, 1111, 1070, 1026, 966, 710 cm^{-1}; HRMS calculated for $[C_{25}{}^{13}C_5H_{49}NO_5 + Na]^+$: 531,3671, found 531,3667.

[1,2,3-$^{13}C_3$]-*N*-Methoxy-*N*-(methyl)-tetradecanamide (25). [1,2,3-$^{13}C_3$]-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 stoppen (~ 2 h), the reaction was concentrated *in vacuo*. The residu 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 lift 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); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.13 (d, *J* = 2.0 Hz, 3 H, $CH_{3\text{-}NMe}$), 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 ($CH_{3\text{-}OMe}$), 32.04 ($CH_{3\text{-}NMe}$), 31.99 (dd, *J* = 51.0, 34.0 Hz, C-2), 29.8-29.3 (m, CH_2 x9), 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 $[C_{13}{}^{13}C_3H_{33}NO_2 + H]^+$: 275.2612, found 275.2683.

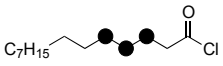
Ethyl (E)-[3,4,5-$^{13}C_3$]-hexadec-2-enoate (27). [1,2,3-$^{13}C_3$]-*N*-(Methoxy)-*N*-Methyl-tetradecanamide **25** (2.74 g, 10.0 mmol, 1.0 eq) was dissolved in dry THF (20 mL) and cooled to -78 °C, before addition of DIBAL-H (1.5 M in toluene) (8.0 ml, 12.0 mmol, 1.2 eq). The reaction was stirred 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_2), filtered and concentrated *in vacuo* giving crude [1,2,3-$^{13}C_3$]-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 mL, 1.25 eq). The reaction was stirred for 10 min at 0 °C. The crude [1,2,3-$^{13}C_3$]-tetradecanal was dissolved in anhydrous in 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); ^{1}H 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, $CH_{2\text{-}Ethyl}$), 2.19 (dm, 2 H, *J* = 126.0 Hz), 1.62-1.22 (m, 25 H, $CH_{3\text{-}Ethyl}$ 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 ($CH_{2\text{-}Ethyl}$), 32.33 (dd, *J* = 41.0, 34.0 Hz, C-4), 29.8-29.0 (m, CH_2 x9), 28.1 (dd, *J* = 34.0, 2.0 Hz, C-5), 22.8 (CH_2), 14.4 ($CH_{3\text{-}Ethyl}$), 14.3 (C-16); IR (neat): 2922, 2852, 1720, 1626, 1466, 1365, 1301, 1263, 1175, 1034, 977, 721 cm^{-1}; HRMS calculated for $[C_{15}{}^{13}C_3H_{34}O_2 + H]^+$: 286.2738, found 286.2733.

Ethyl-[3,4,5-$^{13}C_3$]-hexadecanoate (28). Ethyl (*E*)-[3,4,5-$^{13}C_3$]-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 colomn 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); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (q, 2 H, *J* = 7.2 Hz, $CH_{2\text{-}Ethyl}$), 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, $CH_{3\text{-}Ethyl}$ 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 ($CH_{2\text{-}Ethyl}$), 32.1 (C-2), 29.8-28.8 (m, C-4, C-5 and CH_2 x10), 25.4-24.7 (m, C-3), 22.8 (CH_2), 14.3 ($CH_{3\text{-}Ethyl}$), 14.2 (C-16); IR (neat): 2920, 2851, 1738, 1463, 1238, 1174, 1035, 733 cm^{-1}; HRMS calculated for $[C_{15}{}^{13}C_3H_{36}O_2 + H]^+$: 288.2894, found 288.2889.

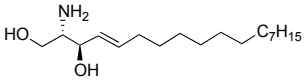
[3,4,5-$^{13}C_3$]-palmitic acid (29). Ethyl-[3,4,5-$^{13}C_3$]-hexadecanoate **28** (2.10 g, 7.30 mmol, 1.0 eq) was dissolved in THF:EtOH:H_2O (1:1:1) (35 mL) and lithium hydroxide (0.52 g, 21.9 mmol, 3.0 eq) was added. The reaction was stirred at room temperature over night. The reaction mixture was 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 (Na_2SO_4), 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); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.1 (COOH), 32.1 (C-2), 29.9-28.7 (m, C-4, C-5 and CH_2 x10), 25.2-24.4 (m, C-3), 22.8 (CH_2), 14.3 (C-16); IR (neat); 2912, 2847, 1694, 1470, 1430, 1308, 1288, 941, 718, 679 cm^{-1}.

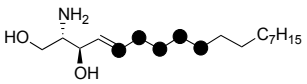
[3,4,5-$^{13}C_3$]-palmitoyl chloride (30). [3,4,5-$^{13}C_3$]-Palmatic acid (1.80 g, 6.93 mmol, 1.0 eq) was dissolved in dry DCM (72 mL), put under an atmosphere of argon, and cooled to 0 °C. Oxalyl chloride (1.2 mL, 14 mmol, 2 eq) was added followed by a drop of DMF. The reaction was then keept under 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4 (C=O), 32.0 (CH_2), 29.8-28.0 (m, C-4, C-5 and 8x CH_2), 25.15 (C-2), 24.14 (dd, *J* = 32.0, 1.5 Hz, C-3), 22.8 (CH_2), 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*-butyloxycarbonyl)-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 producere for HPLS-MS purifications) produced the title compound (41 mg, 0.1 mmol, 54%) as a TFA adduct. $[\alpha]_D^{22}$: -2.0 (c = 0.5 MeOH); ^{1}H 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); ^{13}C NMR (151 MHz, MeOD-d_4) δ 136.6 (C-5), 128.5 (C-4), 71.0 (C-3), 59.4 (C-1), 58.5 (C-2), 33.4 (C-4), 33.1, 30.81, 30.80 (2x CH_2), 30.77, 30.75, 30.65, 30.5, 30.4 (9x CH_2), 30.2 (C-7), 23.8 (CH_2), 14.4 (C-18); IR (neat): 3289, 2918, 2850, 1668, 1520, 1470, 1192, 1134, 986, 720 cm^{-1}; HRMS calculated for $[C_{18}H_{37}NO_2 + H]^+$: 300.2897, found 300.2899.

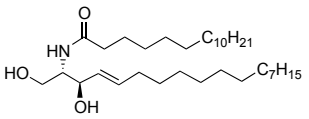


[5,6,7,8,9-$^{13}C_5$]-D-*erythro*-sphingosine (31b). [5,6,7,8,9-$^{13}C_5$]-3-*O*-Benzoyl-*N*-(*tert*-butoxycarbonyl)-D-*erythro*-sphingosine **23b** (90 mg, 0.29 mmol, 1 eq.) was dissolved in methanol (10 mL) and sodium methoxide (30% in methanol) (19 µL, 0.14 mmol, 0.5 eq) was added. The reaction 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. $[\alpha]_D^{22}$: -2.0 (c = 0.5 MeOH); ^{1}H 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); ^{13}C NMR (151 MHz, MeOD-d_4) δ 135.6 (dd, *J* = 42.0, 3.0 Hz, C-5),

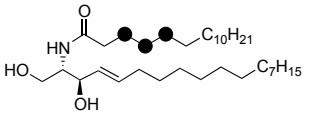


128.5 (dd, *J* = 72.0, 3.0 Hz), 71.0 (dd, *J* = 5.5, 1.3 Hz, C-3), 59.5 (C-1), 58.5 (d, *J* = 3.0 Hz, C-2), 33.8-32.9 (m, C-6 and CH_2), 30.9-29.8 (m, CH_2 x10), 23.7 (CH_2), 14.5 (C-18); IR (neat): 3287, 2914, 2847, 1661, 1526, 1470, 1198, 1136, 966, 721 cm^{-1}; HRMS calculated for $[C_{13}{}^{13}C_5H_{37}NO_2 + H]^+$: 305.2897, found 305.3065.

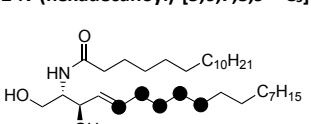
Ceramide (32a). See general procedure for the synthesis of the ceramides from sphingosine. Yield (20 mg, 37 µmol, 79%). R_f = 0.48 (EtOAc:pentane 1:1); $[\alpha]_D{}^{22}$: -7.6 (c = 1.0 MeO:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.26 (d, 1 H, *J* = 7.8 Hz, NH), 5.78 (dt, 1 H, *J* = 15.4, 7.0 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-1_a), 3.90 (m, 1 H, H-2), 3.70 (dd, 1 H, *J* = 11.4, 3.6 Hz, H-1_b), 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*); ^{13}C NMR (150 MHz, $CDCl_3$) 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 (CH_2 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 $[C_{34}H_{67}NO_3 + H]^+$: 538.5121, found 538.5192.



2-*N*-([3,4,5-$^{13}C_3$]-hexadecanoyl)-sphingosine (32b). See general procedure for the synthesis of the ceramides from sphingosine. Yield (14 mg, 25 µmmol, 71%). R_f = 0.48 (EtOAc:pentane 1:1); $[\alpha]_D{}^{22}$: -8.0 (c = 0.1 MeOH:$CHCl_3$ 1:1) ; ^{1}H NMR (600 MHz, $CDCl_3$/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-1_a), 3.90 (m, 1 H, H-2), 3.70 (dd, 1 H, *J* = 11.4, 3.6 Hz, H-1_b), 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*); ^{13}C NMR (151 MHz, $CDCl_3$/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 (CH_2 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 $[C_{31}{}^{13}C_3H_{67}NO_3 + H]^+$: 541.5121, found 541.5293.



2-*N*-(hexadecanoyl)-[5,6,7,8,9-$^{13}C_5$]-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:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) 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-1_a), 3.90 (m, 1 H, H-2), 3.70 (m, 1 H, H-1_a), 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*); ^{13}C NMR (151 Hz, $CDCl_3$) 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 CH_2), 29.9-29.0 (m), 28.25-27.74 (m), 25.92 (m), 22.84 (CH_2 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 $[C_{29}{}^{13}C_5H_{67}NO_3 + H]^+$: 543.5121, found 543.5358.



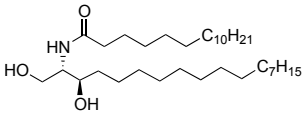
2-*N*-([3,4,5-$^{13}C_3$]-hexadecanoyl)-[5,6,7,8,9-$^{13}C_5$]-sphingosine (32d). See general procedure for the synthesis of the ceramides from sphingosine. Yield (18 mg, 33 µmol, 81%). R_f = 0.48 (EtOAc:pentane 1:1); $[\alpha]_D{}^{22}$: -7.0 (c = 0.33 MeOH:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) 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-1_a), 3.90 (m, 2 H, H-2), 3.70 (dd, 1 H, *J* = 11.4, 3.2 Hz, H-1_b), 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*); ^{13}C NMR(151 MHz, $CDCl_3$) 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 CH_2), 29.9-29.0 (m), 28.2-27.7 (m), 26.2-25.6 (m), 21.7 (CH_2 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 $[C_{26}{}^{13}C_8H_{67}NO_3 + H]^+$: 546.5121, found 546.5461.

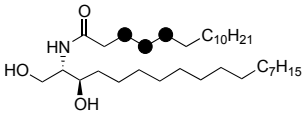
Sphinganine (33a). 3-*O*-Benzoyl-*N*-(tert-butyloxycarbonyl)-D-*erythro*-sphingosine **23a** (36.8 mg, 0.07 mmol, 1.0 eq), was dissolved in methanol (2.4 mL), and sodium methoxide (30% in methanol) (4.6 µL, 0.035 mmol, 0.5 eq) was added. The reaction was stirred at room temperature until TLC showed full 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); ^{1}H 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); ^{13}C 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 (CH_2 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 $[C_{18}H_{39}NO_2 + H]^+$: 302,2981, found 302.3054.

[5,6,7,8,9-$^{13}C_5$]-Sphinganine (33b). [5,6,7,8,9-$^{13}C_5$]-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); ^{1}H 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, 40 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); ^{13}C 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 (CH_2), 31.1-30.2 (m, CH_2 x10), 27.3-26.7 (m, CH_2), 23.8 (CH_2), 14.5 (C-18); IR (neat): 3120, 2914, 2847, 1676, 1206, 1186, 1153, 1130, 840, 800, 723 cm^{-1}; HRMS calculated for $[C_{18}H_{39}NO_2 + H]^+$: 307.2981, found 307.3222.

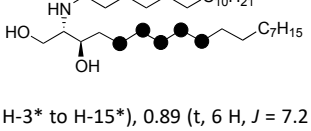
Dihydroceramide (34a). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (12 mg, 22 µmol, 68%); R_f = 0.50 (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: +4.5 (c = 0.15 MeOH:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$, 318 ^{o}K) δ 6.36 (d, 1 H, *J* = 7.6 Hz, NH), 4.01 (d, 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 H15*), 0.88 (t, 6 H, *J* = 7.2 Hz, H-18 and H-16*); ^{13}C NMR (151 MHz, $CDCl_3$, 318 ^{o}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 (CH_2 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 $[C_{34}H_{67}NO_3 + H]^+$: 540.5121, found 540.5347.



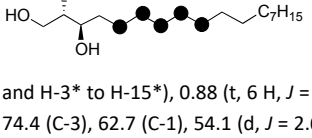
2-*N*-([3,4,5-$^{13}C_3$]-hexadecanoyl)-sphinganine (34b). See general procedure for the synthesis of the ceramides from the sphingosine (15 mg, 27 µmol, 74%); R_f = 0.50 (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: +4.8 (c = 0.1 MeOH:$CHCl_3$ 1:1) ; ^{1}H NMR (600 MHz, $CDCl_3$, 318 ^{o}K) δ 6.26 (d, 1 H, *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, $CDCl_3$, 318 ^{o}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 (CH_2 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 $[C_{31}{}^{13}C_3H_{67}NO_3 + H]^+$: 543.5121, found 543.5442.



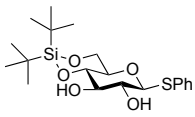
2-*N*-(hexadecanoyl)-[5,6,7,8,9-$^{13}C_5$]-sphinganine (34c). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (14 mg, 25 µmol, 65%); R_f = 0.50 (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: +5.2 (c = 0.25 MeOH:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$, 318 ^{o}K) δ 6.26 (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, $CDCl_3$, 318 ^{o}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 (CH_2 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 $[C_{29}{}^{13}C_5H_{67}NO_3 + H]^+$: 545.5121, found 545.5515.



2-*N*-([3,4,5-$^{13}C_3$]-hexadecanoyl)-[5,6,7,8,9-$^{13}C_5$]-sphinganine (34d). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (18 mg, 33 µmol, 66%); R_f = 0.50 (EtOAc:pentane 1:1); $[\alpha]_D^{22}$: +5.0 (c = 0.25 MeOH:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$, 318 ^{o}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, $CDCl_3$, 318 ^{o}K) δ 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 (CH_2 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 $[C_{26}{}^{13}C_8H_{67}NO_3 + H]^+$: 548.5121, found 548.5611.



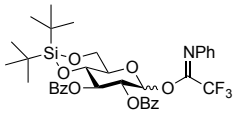
Phenyl-4,6-*O*-(di-*tert*-butylsilanediyl)-1-thio-β-D-glucosylpyranoside (35b). Phenyl-1-thio-β-D-glucoside (**35a**) (1.85 g, 6.8 mmol, 1.05 eq) was dissolved in dry DMF (27 mmol) under an Argon atmosphere. This solution was cooled down to -40 ^{0}C before drop wise addition of di-*tert*-butylsilylbis(trifluoromethanesulfonate) (2.1 mL, 6.5 mmol, 1 eq). The resulting reaction was stirred at -40 ^{o}C for 30 minutes followed by addition of pyridine (1.58 mL,

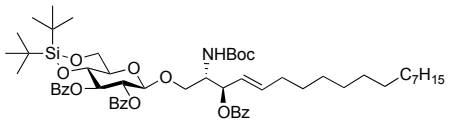


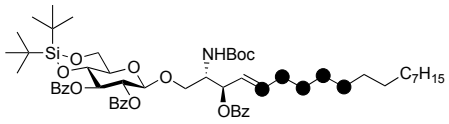
19.5 mmol, 3 eq). The reaction was stirred for an additional 15 minutes and then transferred to an extraction funnel with diethyl ether (50 mL). The organics were washed with water (2x 100 mL) and brine (100 mL). The aqueous layers were extracted with diethyl ether (50 mL) and the combined organics were dried (Na_2SO_4) filtered and concentrated *in vacuo*. Purification by silica column chromatography (10% Et_2O 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 $CHCl_3$); ^{1}H 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-$_{tBu\text{-}Si}$), 0.98 (s, 3 H, H-$_{tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.9 ($C_{q\text{-}arom}$), 131.9 ($C_{q\text{-}arom}$), 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 ($CH_{3\text{-}tBu\text{-}Si}$), 27.1 ($CH_{3\text{-}tBu\text{-}Si}$), 22.8 ($C_{q\text{-}tBu\text{-}Si}$), 20.0 ($C_{q\text{-}tBu\text{-}Si}$); IR (neat): 3380, 2931, 2858, 1472, 1055, 823, 731, 651 cm^{-1}; HRMS: calculated. For $[C_{20}H_{32}O_5SSi + H]^+$ 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 gelcolumn chromatography (10% Et_2O 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); ^{1}H 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, $CH_{3\text{-}tBu\text{-}Si}$), 0.967 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$); ^{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 ($CH_{3\text{-}tBu\text{-}Si}$), 26.9 ($CH_{3\text{-}tBu\text{-}Si}$), 22.5 ($C_{q\text{-}tBu\text{-}Si}$), 19.9 ($C_{q\text{-}tBu\text{-}Si}$); IR (neat): 2959, 2932, 2883, 2858, 1732, 1271, 1177, 1126, 827, 708 cm^{-1}; HRMS: calculated for $[C_{34}H_{40}O_7SSi + H]^+$ 621.2344; found 621.2337.

2,3-di-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilaanediyl)-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*-iodosuccamide (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); ^{1}H 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 ($CH_{3\text{-}tBu\text{-}Si}$), 27.41 ($CH_{3\text{-}tBu\text{-}Si}$), 27.00 ($CH_{3\text{-}tBu\text{-}Si}$), 26.96 ($CH_{3\text{-}tBu\text{-}Si}$), 22.75 ($C_{q\text{-}tBu\text{-}Si}$), 22.72 ($C_{q\text{-}tBu\text{-}Si}$), 20.08 ($C_{q\text{-}tBu\text{-}Si}$), 20.05($C_{q\text{-}tBu\text{-}Si}$); IR (neat): 3431, 2934, 2859, 1728, 1277, 1177, 1070, 827, 708 cm^{-1}; HRMS: calculated for $[C_{26}H_{38}O_8Si + H]^+$ 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 (35). 
2,3-di-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilaanediyl)-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-trifluoroimidiate (0.78 mL, 5.18 mmol, 1.5 eq), and the reaction was stirred at 0 ^{0}C for 2 hours. The reaction mixture was filtered and concentrated *in vacuo*. Purifiaction by silica gel column chromatography, using silica gel that was neutralized by running an eluent of 3% Et_3N in petroleum ether (100 mL) through the column (0-5% EtOAc, 20% DCM in petroleum ether) produced the title compound (1.93 g, 2.76 mmol, 80%,). R_f = 0.1 (10% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) 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}); ^{13}C NMR (101 MHz, $CDCl_3$) 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 ($C_{q\text{-}tBu\text{-}Si}$), 26.83 ($C_{q\text{-}tBu\text{-}Si}$), 22.65 ($C_{q\text{-}tBu\text{-}Si}$), 20.00 ($C_{q\text{-}tBu\text{-}Si}$); IR (neat): 2959, 2936, 2860, 1728, 1273, 1211, 995, 766, 710 cm^{-1}; HRMS: calculated for $[C_{36}H_{40}F_3NO_8Si + H]^+$ 700.2555, found 700.2549.

Glucosyl sphingosine (36a). 
2,3-di-*O*-Benzoyl-4,6-*O*-(di-*tert*-butylsilanediyl)-1-*O*-(*N*-[phenyl]-trifluoracetimidoyl) - 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 $BF_3{\cdot}OEt_2$ (44 µL, 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. $NaHCO_3$ (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 column chromatography (2-5% Et_2O, 20% DCM in petroleum ether) produced the title compound as an amorphous solid (177 mg, 0.17 mmol, 49%); R_f = 0.45 (10% Et_2O, 20% DMC in petroleum ether); $[\alpha]_D^{22}$: +6.8 (c = 0.1 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 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-$1_{a\text{-}Sp}$ 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-$1_{b\text{-}Sp}$), 3.55 (m, 1 H, H-5), 1.96 (q, 2 H, *J* = 6.8 Hz, H-6_{sp}), 1.34 (s, 9 H, $CH_{3\text{-}tBu\text{-}Boc}$), 1.32-1.18 (m, 22 H, H-7_{sp} to H-17_{Sp}), 0.95 (s, 18 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18_{sp}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 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 ($C_{q\text{-}arom}$), 129.9 (CH_{arom} x2), 129.8 ($C_{q\text{-}arom}$), 129.7 (CH_{arom} x4), 129.5 ($C_{q\text{-}arom}$), 128.5 (CH_{arom} x2), 128.4 (CH_{arom} x4), 124.7 (C-4_{sp}), 101.4 (C-1), 79.5 ($C_{q\text{-}Boc}$), 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 ($CH_{2\text{-}Sp}$ x10), 28.4 ($CH_{3\text{-}tBu\text{-}Boc}$), 27.4, 26.9 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 22.8 ($C_{q\text{-}tBu\text{-}Si}$), 22.6 ($CH_{2\text{-}Sp}$), 20.0 ($C_{q\text{-}tBu\text{-}Si}$), 14.3 (C-$18_{Sp}$); IR (neat): 3070, 2958, 2924, 2854, 1728, 1271, 1174, 1103, 1070, 709 cm^{-1}; HRMS calculated for $[C_{58}H_{83}NO_{12}Si + Na]^+$: 1036.5685, found 1036.5584.

[5,6,7,8,9-$^{13}C_5$]-glucosyl sphingosine (36b). 
2,3-di-*O*-Benzoyl-4,6-*O*-(di-*tert*-butylsilanediyl)-1-*O*-(*N*-[phenyl]-trifluoracetimidoyl)-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 molsievies (3 Å) were added and the mixture was stirred for 1 h at room temperature and then cooled to 0 °C, before addition of $BF_3{\cdot}OEt_2$ (35 µL, 0.27 mmol,

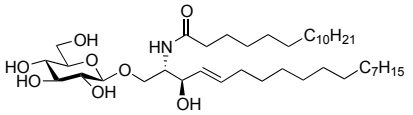
1.0 eq). The reaction was stirred until TLC showed a lower running spot (debocylation of sphingosine acceptor) (~ 1 h). The reaction mixture was transferred to an extraction funnel with EtOAc (40 mL), and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (2-5% Et_2O, 20% DCM in petroleum ether) produced the title compound as an amorphous solid (147 mg, 0.145 mmol, 54%). R_f = 0.45 (10% Et_2O, 20% DMC in petroleum ether); $[\alpha]_D^{22}$: +6.0 (c = 0.1 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 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-$1_{a\text{-}Sp}$ 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-$1_{b\text{-}Sp}$), 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, $CH_{3\text{-}tBu\text{-}Boc}$ and H-7_{sp} to H-17_{Sp}), 0.95 (s, 18 H, $CH_{3\text{-}tBu\text{-}Si}$), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18_{Sp}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 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 ($C_{q\text{-}arom}$), 129.9 (CH_{arom} x2), 129.8 ($C_{q\text{-}arom}$), 129.7 (CH_{arom} x4), 129.5 ($C_{q\text{-}arom}$), 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 ($C_{q\text{-}Boc}$), 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 ($CH_{2\text{-}Sp}$), 29.80-28.4 (m, $CH_{2\text{-}Sp}$ x9 and $CH_{3\text{-}tBu\text{-}Boc}$), 27.4, 26.9 ($CH_{3\text{-}tBu\text{-}Si}$ x2), 22.8 ($C_{q\text{-}tBu\text{-}Si}$), 22.6 ($CH_{2\text{-}Sp}$), 20.0 ($C_{q\text{-}tBu\text{-}Si}$), 14.3 (C-$18_{Sp}$); IR (neat): 3070, 2922, 2854, 1724, 1267, 1172, 1069, 827, 708 cm^{-1}; HRMS calculated for $[C_{53}{}^{13}C_5H_{83}NO_{12}Si + H]^+$: 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. $NaHCO_3$ (30 mL), and brine (30 mL). The aqueous layers were extracted with EtOAc (30 mL) and the combined organics were dried (Na_2SO_4), 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 reactio was then quenched with AcOH (0.73 mL, 13 mmol, 100 eq) and concentrated *in vacuo*. The crude reaction mixture was coevaporated in tolune 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. $[\alpha]_D^{22}$: -5.0 (c = 0.1 MeOH); ^{1}H 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-$1_{a\text{-}Sp}$), 3.66 (m, 1 H, H-$1_{b\text{-}Sp}$), 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, 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 $CH_{2\text{-}Sp}$), 14.2 (C-18_{Sp}); IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm^{-1}; HRMS calculated for $[C_{24}H_{47}NO_7 + H]^+$: 462.3431, found 462.3424.

Glucosyl-[5,6,7,8,9-$^{13}C_5$]-Sphingosine (37b). Protected glucosyl-[5,6,7,8,9-$^{13}C_5$]-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. $NaHCO_3$ (20 mL), and brine (20 mL). The

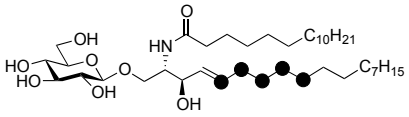
aqueous layers were extracted with EtOAc (20 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude mixture was dissolved in MeOH (8 mL) and sodium methoxide (30% in methanol) (6.5 µL, 47 µmol, 1.0 eq) was added. The reaction was stirred over night at room 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); ^{1}H NMR (600 MHz, 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$_{a\text{-}Sp}$), 3.66 (dd, 1 H, *J* = 11.7, 6.1 Hz, H-1$_{b\text{-}Sp}$), 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, 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 CH$_{2\text{-}Sp}$), 30.9-29.6 (m, CH$_{2\text{-}Sp}$ x10), 23.4 (CH$_{2\text{-}Sp}$), 14.5 (C-18$_{Sp}$); IR (neat): 3300, 2918, 2851, 1670, 1433, 1200, 1134, 1074, 1024, 800, 721 cm^{-1}; HRMS calculated for $[C_{19}{}^{13}C_5H_{47}NO_7 + H]^+$: 467.3598, found 467.3591.

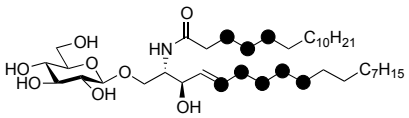
Glucosylceramide (38a). See general procedure for the synthesis of the ceramides from the sphingosine. Yield

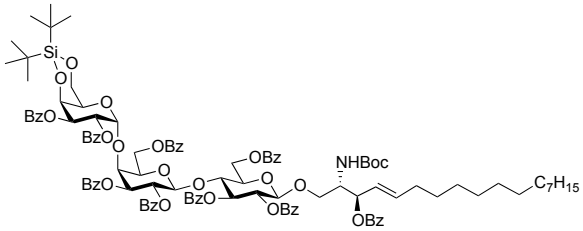


(3.1 mg, 4.4 µmol, 57%); R_f = 0.25 ($CHCl_3$:MeOH 9:1); $[\alpha]_D^{22}$: +6.0 (c = 0.1 MeOH:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$/MeOD-*d*$_4$) δ 5.68 (dt, 1 H, *J* = 13.8, 6.9 Hz, H-5$_{Sp}$), 5.44 (dd, 1 H, *J* = 15.3, 7.8 Hz, H-4$_{Sp}$), 4.26 (d, 1 H, *J* = 7.9 Hz, H-1'), 4.16 (dd, 1 H, *J* = 10.3, 4.8 Hz, H-1$_{b\text{-}Sp}$), 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$_{a\text{-}Sp}$), 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, $CDCl_3$/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 (CH$_2$ 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 $[C_{40}H_{77}NO_8 + H^+]$: 700.5727, found 700.5720.

Glucosyl-2-*N*-([3,4,5-^{13}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:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, 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$_{b\text{-}Sp}$), 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$_{a\text{-}Sp}$), 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, 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 (CH$_2$ 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 $[C_{37}{}^{13}C_3H_{77}NO_8 + H^+]$: 703.5828, found 703.5821.

Glucosyl-2-*N*-(hexadecanoyl)-[5,6,7,8,9-$^{13}C_5$]-sphingosine (38c). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (2.8 mg, 3.9 µmol **,** 52%).  R_f = 0.25 ($CHCl_3$:MeOH 9:1); $[\alpha]_D^{22}$: +5.4 (c = 0.1 MeOH:$CHCl_3$); ^{1}H 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$_{b\text{-}Sp}$), 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$_{a\text{-}Sp}$), 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*); ^{13}C 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 (CH_2 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 [$C_{35}{}^{13}C_5H_{77}NO_8$ + H$^+$]: 705.5895, found 705.5906.

Glucosyl-2-*N*-([3,4,5-$^{13}C_3$]-hexadecanoyl)-[5,6,7,8,9-$^{13}C_5$]-sphingosine (38d). See general procedure for the synthesis of the ceramides from the sphingosine. Yield (4.9 mg, 6.9 µmol, 61%);  R_f = 0.25 ($CHCl_3$:MeOH 9:1); $[\alpha]_D^{22}$: +5.0 (c = 0.2 MeOH:$CHCl_3$ 1:1); ^{1}H 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$_{b\text{-}Sp}$), 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$_{a\text{-}Sp}$), 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*); ^{13}C 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 (CH_2 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 [$C_{32}{}^{13}C_8H_{77}NO_8$ + 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 molsieves (3 Å) were added and the mixture was stirred for one hour at room temperature and then cooled to 0 ºC, before addition of $BF_3{\cdot}OEt_2$ (48% in Et_2O) (38 µL, 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. $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by column chromatography (12% Et_2O, 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% Et_2O, 20% DCM in petroleum ether); $[\alpha]_D^{22}$: +31 (c = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 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$_{a\text{-}Sp}$ and H-2$_{Sp}$),

3.97 (dd, 1 H, *J* = 10.9, 5.3 Hz, H-6$_a$'), 3.81-3.72 (m, 2 H, H-5 and H-6$_b$'), 3.59 (m, 1 H, H-1$_{b\text{-}Sp}$), 3.53 (m, 1 H, H-5'), 1.88 (m, 2 H, H-6$_{Sp}$), 1.33 (s, 9 H, CH$_{3\text{-}t\text{Bu-Boc}}$), 1.30-1.11 (m, 22 H, H-7$_{Sp}$ to H-17$_{Sp}$), 1.06 (s, 9 H, CH$_{3\text{-}t\text{Bu-Si}}$), 1.00 (s, 9 H, CH$_{3\text{-}t\text{Bu-Si}}$), 0.87 (t, 3 H, *J* = 6.8 Hz, H-18$_{Sp}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 165.9, 165.7, 165.6, 165.2, 164.98, 164.95, 164.8, 164.7 (C=O$_{Bz}$ x9), 155.2 (C=O$_{Boc}$), 137.2 (C-5$_{sp}$), 133.4, 133.13, 133.10, 132.97, 132.94, 132.87, 132.7 (CH$_{arom}$ x7), 130.2 (C$_{q\text{-}arom}$), 130.1, 130.0 (CH$_{arom}$ x2), 129.9 (C$_{q\text{-}arom}$), 129.8, 129.64, 129.59, 129.58, 129.5 (CH$_{arom}$ x5), 129.4 (C$_{q\text{-}arom}$), 129.3 (CH$_{arom}$), 129.2, 129.0, 128.6, 128.50, 128.47 (C$_{q\text{-}arom}$ x5), 128.45, 128.39, 128.35, 128.29, 128.28, 128.16, 128.12, 128.07 (CH$_{arom}$ x8), 124.4 (C-4$_{Sp}$), 101.3 (C-1'), 100.9 (C-1), 98.68 (C-1''), 79.3 (C$_{q\text{-}Boc}$), 76.6 (C-4), 76.3 (C-4'), 74.3 (C-3$_{Sp}$), 73.02 (C-5), 72.94 (C-3), 72.8 (C-3'), 72.6 (C-5'), 71.9 (C-2), 71.2 (C-3''), 71.0 (C-4''), 69.7 (C-2'), 69.5 (C-2''), 68.3 (C-5''), 67.8 (C-1$_{Sp}$), 66.9 (C-6'), 62.3 (C-6), 60.5 (C-6''), 52.3 (C-2$_{Sp}$), 32.2 (C-6$_{Sp}$), 31.9, 29.62 (x3), 29.61, 29.5, 29.3, 29.2, 28.7 (CH$_{2\text{-}Sp}$ x10), 28.2 (CH$_{3\text{-}t\text{Bu-Boc}}$), 27.5, 27.2 (CH$_{3\text{-}t\text{Bu-Si}}$ x2), 23.2 (C$_{q\text{-}t\text{Bu-Boc}}$), 22.6 (CH$_{2\text{-}Sp}$), 20.7 (C$_{q\text{-}t\text{Bu-Si}}$), 14.1 (C-18$_{Sp}$); IR (neat): 3070, 2926, 2856, 1722, 1451, 1267, 1095, 1070, 1028, 708 cm^{-1}; HRMS calculated for [$C_{112}H_{127}NO_{28}Si$ + Na]$^+$: 1984.8206, found 1984.8204.

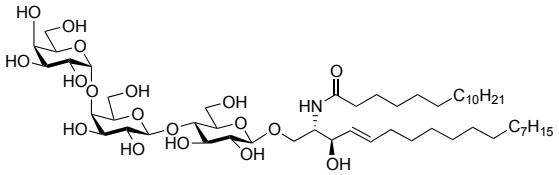
[5,6,7,8,9-$^{13}C_5$]-Globotraiosyl sphingosine (40b). Globotriaosyl imidate donor **(39)** (158 mg, 96 µmol, 1.2 eq) and $^{13}C_5$-sphingosine acceptor **23b** (40.7 mg, 80 µmol, 1.0 eq) were co-evaporated twice with toluene (5 mL) and then dissolved in anhydrous DCM (2 mL). Activated molsieves (3 Å) were added and the mixture was stirred at room temperature for 1 hour and then cooled to 0 ºC, before addition of $BF_3{\cdot}OEt_2$ (48% in Et_2O) (23 µL, 88 µmol, 1.1 eq). The reaction was stirred until TLC showed complete conversion of the $^{13}C_5$-sphingosine acceptor (~2 h). The reaction mixture was then transferred to an extraction funnel with EtOAc (40 mL) and washed with sat. aq. $NaHCO_3$ (40 mL) and brine (30 mL). The aqueous layers were then extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography (12% ether, 10% DCM in petroleum ether) **40b** as an amorphous solid (87 mg, 44 µmol, 55%). R_f = 0.54 (30% ether, 20% DCM in petroleum ether); $[\alpha]_D^{22}$: +30 (c = 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (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$_{a\text{-}Sp}$ and H-2$_{Sp}$), 3.97 (dd, 1 H, J = 10.9, 5.4 Hz, H-6$_a$'), 3.81 – 3.71 (m, 2 H, H-5 and H-6$_b$'), 3.58 (m, 1 H, H-1$_{b\text{-}Sp}$), 3.51 (dd, 1 H, J = 13.9, 6.9 Hz, H-5'), 1.87 (dm, 2 H, J = 124.6 Hz, H-6$_{Sp}$), 1.40 – 1.14 (m, 31 H, H-7$_{Sp}$ to H-17$_{Sp,}$ and CH$_{3\text{-}t\text{Bu-Boc}}$), 1.05 (s, 9 H, CH$_{3\text{-}t\text{Bu-Si}}$), 1.00 (s, 9 H, CH$_{3\text{-}t\text{Bu-Si}}$), 0.87 (t, 3 H, J = 6.8 Hz, H-18$_{Sp}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 166.0, 165.7, 165.6, 165.2, 165.00, 164.98, 164.79, 164.75 (C=O$_{Bz}$ x9), 155.2 (C=O$_{Boc}$), 137.2 (d, J = 42.4 Hz, C-5$_{Sp}$), 133.4, 133.2, 133.1, 133.00, 132.99, 132.96, 132.89, 132.7, 130.2, 130.1, 130.00, 129.95, 129.8, 129.67, 129.62, 129.60, 129.50, 129.48, 129.4, 129.2, 129.0, 128.6, 128.53, 128.50, 128.48, 128.41, 128.37, 128.32, 128.30, 128.18, 128.14, 128.09 (CH$_{arom}$ and C$_{q\text{-}arom}$ x32), 124.4 (d, J = 71.2 Hz, C-4$_{Sp}$), 101.3 (C-1'), 100.9 (C-1), 98.7 (C-1''), 79.4 (C$_{q\text{-}Boc}$), 76.6 (C-4), 76.3 (C-4'), 74.3 (d, J = 5.4 Hz, C-3$_{Sp}$), 73.04 (C-5), 72.97 (C-3), 72.8 (C-3'), 72.7 (C-5'), 71.9 (C-2), 71.2 (C-3''), 71.0 (C-4''), 69.7 (C-2'), 69.6 (C-2''), 68.3 (C-5''), 67.8 (C-1$_{Sp}$), 66.9 (C-6'), 62.3 (C-6), 60.5 (C-6''), 52.3 (d, J = 2.4 Hz, C-2$_{Sp}$), 32.2 (m, C-6$_{Sp}$), 31.9 (CH$_{2\text{-}Sp}$), 29.8 – 28.1 (m, CH$_{2\text{-}Sp}$ x9 and CH$_{3\text{-}t\text{Bu-Boc}}$), 27.5, 27.2 (CH$_{3\text{-}t\text{Bu-Si}}$ x2), 23.2 (C$_{q\text{-}t\text{Bu-Si}}$), 22.7 (C$_{Sp}$), 20.7 (C$_{q\text{-}t\text{Bu-Si}}$), 14.1 (C-18$_{Sp}$); IR (neat): 3070, 2925, 2853, 1718, 1452, 1266, 1094, 1069, 706 cm^{-1}; HRMS calculated for [$C_{107}{}^{13}C_5H_{127}O_{28}Si$ + 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 µL, 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 µL, 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 trifluoracetic acid (5 mL). The reaction mixture was completely dissolved after one minute and was then stirred for another minute at 0 °C. The solution was then transferred to a round bottom flask containing toluene (50 mL) and concentrated to about 10 mL *in vacuo*. The 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 globotraiosylsphingosine **41a** (43 mg, 54 µmol, 53%) as a TFA adduct. $[\alpha]_D^{22}$: +34.0 (c = 0.5 MeOH); ^{1}H NMR (600 MHz, 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-$1_{a\text{-}Sp}$), 3.94 (dd, 1 H, *J* = 11.9, 2.6 Hz, H-6_a), 3.93-3.91 (m, 2 H, H-4'' and H-$1_{b\text{-}Sp}$), 3.89 (dd, 1 H, *J* = 7.7, 4.1 Hz, H-6_b), 3.88-3.81 (m, 3 H, H-6_a',H-6_b' and H-2''), 3.77 (dd, 1 H, *J* = 10.2, 3.2 Hz, H-3''), 3.74 (dd, 1 H, *J* = 11.1, 7.1 Hz, H-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, MeOD-d_4) δ: 136.8 (C-5_{Sp}), 128.3 (C-4_{Sp}), 105.4 (C-1'), 103.7 (C-1), 102.7 (C-1''), 80.8 (C-4), 79.8 (C-4'), 76.6 (C-5' and C-5), 76.3 (C-2'), 74.7 (C-3), 74.6 (C-2), 72.8 (C-5''), 72.6 (C-3'), 71.3 (C-4''), 71.0 (C-3''), 70.8 (C-3_{Sp}), 70.5 (C-2''), 67.1 (C-1_{Sp}), 62.7 (C-6''), 61.6 (C-6), 61.5 (C-6'), 56.7 (C-2_{Sp}), 33.4 (C-6_{Sp}), 33.1, 30.79 (x3), 30.76, 30.74, 30.6, 30.5, 30.4, 30.2, 23.7 ($CH_{2\text{-}Sp}$ x11), 14.4 (C-18_{Sp}); IR (neat): 3345 bs, 2925, 2855, 1674, 1202, 1134, 1067, 1027, 974, 801, 721 cm^{-1}; HRMS calculated for $[C_{36}H_{67}NO_{17} + H]^+$: 786.4482, found 786.4485.

Globotriaosyl-[5,6,7,8,9-$^{13}C_5$]-sphingosine (41b). Globally protected globotriaosyl-[5,6,7,8,9-$^{13}C_5$]-sphingosine **40b** (87 mg, 0.45 µmol, 1.0 eq) was dissolved in THF:pyridine 4:1 (10 mL) and hydrogen fluoride (70% HF in pyridine) (24 µL, 0.11 mmol, ca. 20 eq) 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 µL, 0.45 µmol, 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

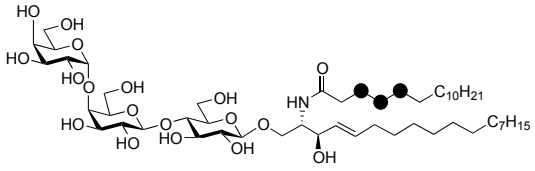
before the addition of trifluoroacetic acid (3 mL). The product was completely dissolved in about 1 min and the reaction was stirred for an additional minute at 0 °C. The solution was then transferred to a round bottom flask containing toluene (50 mL) and concentrated *in vacuo* to about 10 mL. The co-evaporation 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 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 **41b** (17 mg, 21 µmol, 48%) as a TFA adduct. $[\alpha]_D^{22}$: +33.0 (c = 0.20 MeOH); ^{1}H NMR (600 MHz, MeOD-*d*$_4$) δ 5.85 (dm, 1 H, *J* = 150.2 Hz, H-5$_{Sp}$), 5.47 (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$_{a\text{-}Sp}$), 3.94 (dd, 1 H, *J* = 12.0, 2.4 Hz, H-6$_a$), 3.92 – 3.90 (m, 2 H, H-4'' and H-1$_{b\text{-}Sp}$), 3.89 (dd, 1 H, *J* = 7.7, 4.0 Hz, H-6$_b$), 3.88 – 3.79 (m, 3 H, H-6$_a$', H-6$_b$' and H-2''), 3.77 (dd, 1 H, *J* = 10.1, 3.1 Hz, H-3''), 3.74 (dd, 1 H, *J* =11.2, 7.3 Hz, H-6$_a$''), 3.70 – 3.65 (m, 2 H, H-5' and H-6$_b$''), 3.58 – 3.50 (m, 4 H, H-4, H-3, H-3' and H-2'), 3.46 (m, 1 H, H-5), 3.40 (ddd, 1 H, *J* = 8.5, 4.7, 3.6 Hz, H-2$_{Sp}$), 3.30 (m, 1 H, H-2), 2.10 (dm, 2 H, *J* = 126.9 Hz, H-6$_{Sp}$), 1.56 – 1.14 (m, 22 H, H-7$_{Sp}$ to H-17$_{Sp}$), 0.89 (t, 3 H, *J* = 7.0 Hz, H-18$_{Sp}$); ^{13}C NMR (151 MHz, MeOD-*d*$_4$) δ 136.8 (d, *J* = 42.8 Hz, C-5$_{Sp}$), 128.3 (d, *J* = 72.3 Hz, C-4$_{Sp}$), 105.4 (C-1'), 103.7 (C-1), 102.7 (C-1''), 80.8 (C-4), 79.8 (C-4'), 76.6 (C-5' and C-5), 76.3 (C-2'), 74.65 (C-3), 74.2 (C-2), 72.8 (C-5''), 72.6 (C-3'), 71.3 (C-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 $CH_{2\text{-}Sp}$), 30.9 – 29.8 (m, $CH_{2\text{-}Sp}$ x10), 23.7 ($CH_{2\text{-}Sp}$), 14.4 (C-18$_{Sp}$); HRMS calculated for $[C_{31}{}^{13}C_5H_{67}NO_{17}H]^+$: 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 ($CHCl_3$:MeOH:H_2O 70:27:3); $[\alpha]_D^{22}$: +24 (c = 0.75 MeOH:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$/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*); ^{13}C NMR (151 MHz, $CDCl_3$/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 (CH_2 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 $[C_{52}H_{97}NO_{18} + H]^+$: 1024.6784, found 1024.6783.

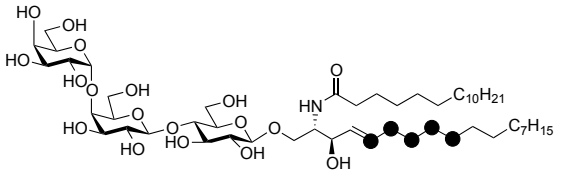
Globotriaosyl-2-*N*-([3,4,5-$^{13}C_3$]-hexadecanoyl)-sphingosine (42b). See general procedure for the synthesis of the ceramides from the sphingosine.



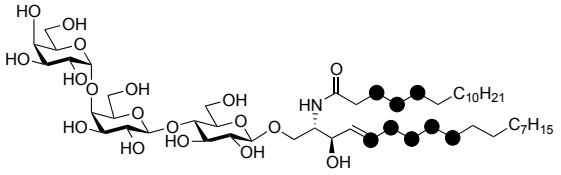
Yield (9 mg, 8.7 µmol, 71%); R_f = 0.35 ($CHCl_3$:MeOH:H_2O 70:27:3); $[\alpha]_D^{22}$: +24 (c = 0.25 (MeOH:$CHCl_3$ 1:1); ^{1}H NMR (600 MHz, $CDCl_3$/MeOD-*d*$_4$) δ 5.69 (dt, 1 H, *J* = 14.7, 6.9 Hz, H-5$_{Sp}$), 5.45 (dd, 1 H, *J* = 15.3, 7.8 Hz, H-4$_{Sp}$), 4.96 (d, 1 H, *J* = 3.8 Hz, H-1''), 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*); ^{13}C NMR (151 MHz, $CDCl_3$/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 (CH_2 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 $[C_{49}{}^{13}C_3H_{97}NO_{18} + H]^+$: 1027.6884, found 1027.6881.

Globotriaosyl-2-*N*-(hexadecanoyl)-[5,6,7,8,9-$^{13}C_5$]-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 ($CHCl_3$:MeOH:H_2O 70:27:3); $[\alpha]_D{}^{22}$: +26 (c = 0.15 MeOH:$CHCl_3$ 1:1); ^{1}H NMR (850 MHz, $CDCl_3$/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*); ^{13}C NMR (213 MHz, $CDCl_3$/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 (CH_2 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 $[C_{47}{}^{13}C_5H_{97}NO_{18} + H]^+$: 1029.6951, found 1029.6949.



Globotriaosyl-2-*N*-([3,4,5-$^{13}C_3$]-hexadecanoyl)-[5,6,7,8,9-$^{13}C_5$]-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 ($CHCl_3$:MeOH:H_2O 70:27:3); $[\alpha]_D{}^{22}$: +25 (c = 0.1 MeOH:$CHCl_3$ 1:1); ^{1}H NMR (850 MHz, $CDCl_3$/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*); ^{13}C NMR (213 MHz, $CDCl_3$/MeOD-d_4) δ 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 (CH_2 x24, C-7_{Sp} to C-17_{Dp} 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 $[C_{44}{}^{13}C_8H_{97}NO_{18} + H]^+$: 1032.7052, found 1032.7053.



2.4 References and notes

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