The synthesis of chemical tools for studying sphingolipid metabolism

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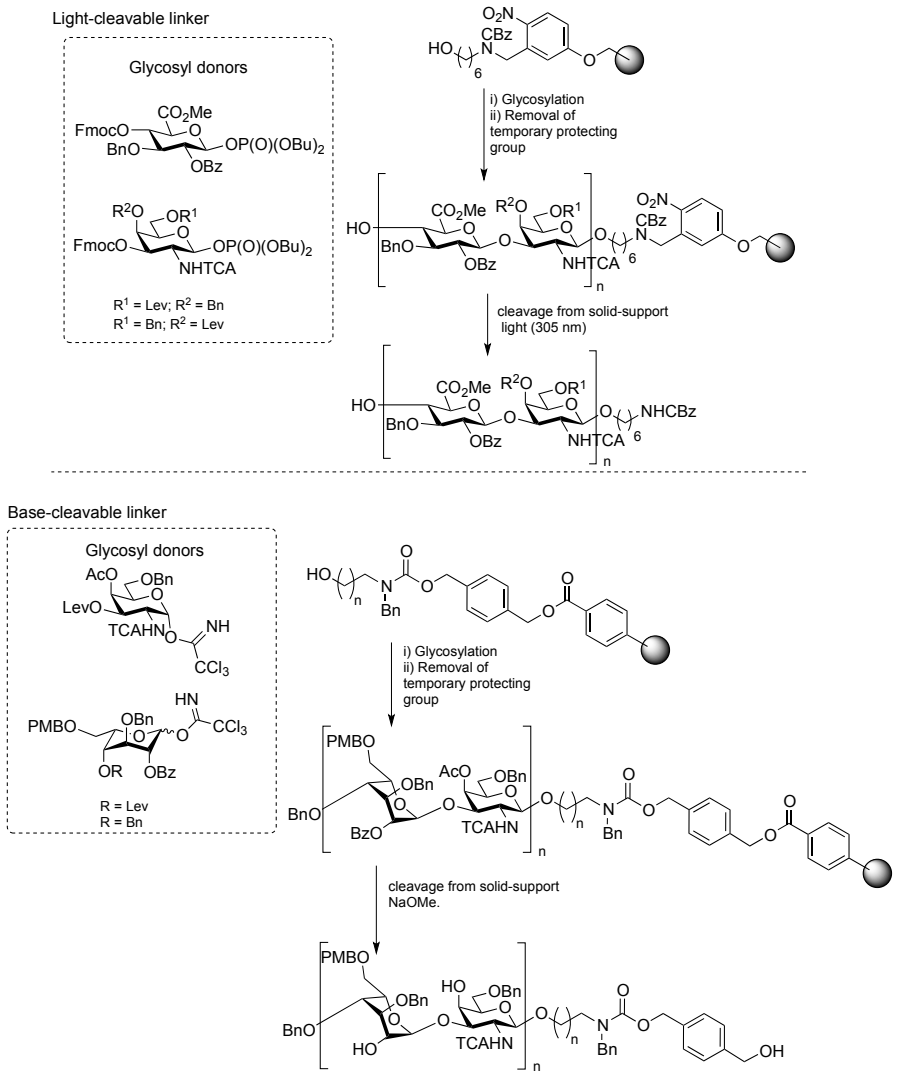
Chapter 7

Summary and Future Prospects

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

in natural abundance and stable-isotope-enriched forms. As well, methodology has been developed that allowed the synthesis of a rare hydroxylated sphingosine base, as well as unnatural, azide-modified sphingoid bases. Although not discussed in depth in this Thesis, the compounds prepared during this PhD work are highly useful in chemical biology and metabolomics studies on biological systems and processes in which sphingolipid metabolism plays a key role. These include human inherited disorders in which a specific enzyme involved in sphingolipid metabolism is impaired, such as the lysosomal storage disorders, Gaucher disease and Fabry disease. **Chapter 1** introduces sphingolipid biology with a focus on the current status in the literature on chemically prepared, sphingolipid-derived reporter molecules by reviewing the literature of existing sphingolipid analogues including those that are isotopically labeled, equipped with a fluorophore, or outfitted with a bioorthogonal ligation handle. **Chapter 2** describes the synthesis of a panel of carbon-13-labeled (glyco-)sphingolipids based on a cross-metathesis event as the key step. The linear, terminal $^{13}C_5$-1-pentadecene for this purpose was assembled from $^{13}C_2$-acetic acid and potassium ^{13}C-cyanide using, amongst other transformations, Horner-Wadsworth-Emmons chemistry. After assembling the partially protected sphingosine base (which was prepared both in $^{13}C_5$-enriched and natural abundance-carbon form), different donor glycosides (glucosyl and GB3) were condensed with the free primary alcohol to yield, after further chemical manipulations, the corresponding glycosylsphingosines. *N*-acylation of the free amine of these with palmitoyl chloride ($^{13}C_0$ or $^{13}C_3$) produced the corresponding glycosylceramides. Although all target compounds could be obtained in good quantity and purity, a caveat of the procedure described in Chapter 2 is the low yield in the glycosylation steps, which can be correlated to the acid-sensitivity of the N-Boc protective group chosen. With the aim to improve on the methodology, **Chapter 3** introduces the use of the Fmoc group, instead of the Boc group, as a means to protect the secondary amine in the sphingoid base. This change of *N*-protective group necessitated also changing the nature of the protective group for the secondary alcohol in the sphingoid base, as is outlined in Chapter 3. The net result of the studies described in Chapter 3 is an improvement in yield in the glycosylation step, and $^{13}C_6$-glucosylsphingosine, 6-azido-6-deoxy-glucosylsphingosine as well as galactosylsphingosine were readily prepared following the new strategy, in good yield and in excellent purity.

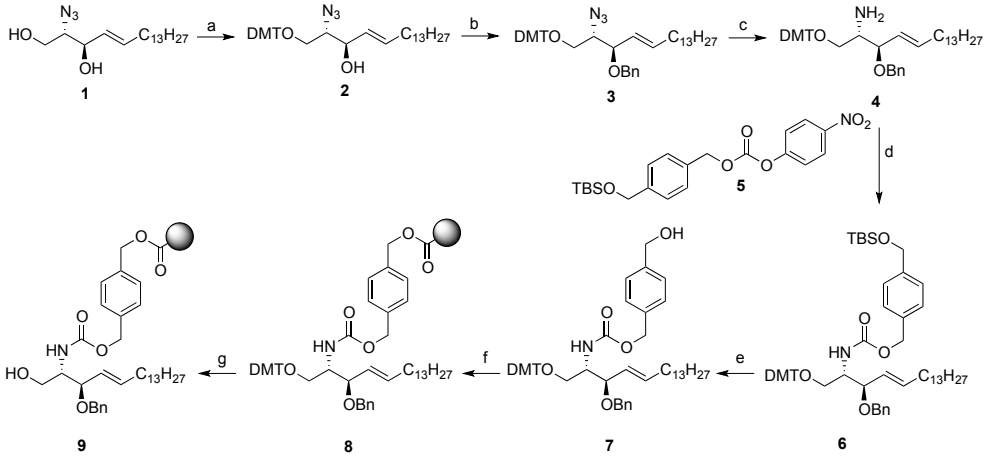
Scheme 7.1 Automated solid-phase oligosaccharide synthesis using light[2]- or base[5] sensitive linkers.



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

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

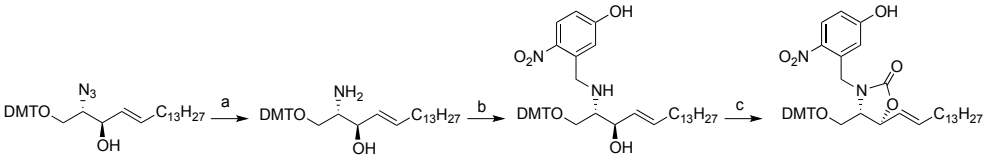
Scheme 7.2 Synthesis of immobilized sphingosine featuring a base-labile linker system.



Reagents and conditions: (a) DMTCl, 2,4,6-collidine, DCM, 0 °C, 2 h, 92%; (b) (i) NaH, DMF, 0 °C, 15 min; (ii) BnBr, TBAI, 0 °C to r.t., 20 h, 88%; (c) (i) PMe_3, wet THF, r.t., 20 h; (d) DIPEA, DMF, 0 °C to r.t., 20 h, 75%; (e) TBAF (1 M in THF), THF, r.t., 2 h, 98%; (f) DIC, DMAP, DCM, 20 h; (g) TCA, DCM, 5 min.

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

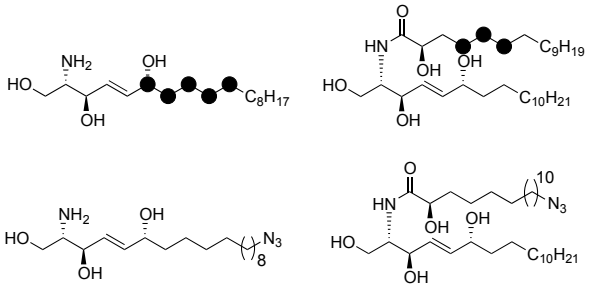
Scheme 7.3 Synthesis light-cleavable sphingosine solid-support.



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

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

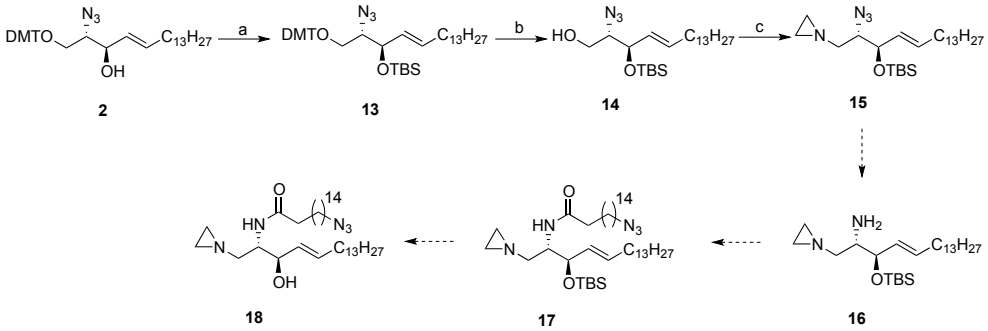
Scheme 7.4 Example of possible modifications of 6-hydroxysphingosines and alpha-hydroxy fatty acids.



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

aziridines into potential two-step activity-based probes. Another interesting ceramide-mimetic aziridine is compound **18**, in which the primarily hydroxyl has been substituted by an aziridine (Scheme 7.5). The synthesis of **18** started from DMT-protected sphingosine **2**, which was silylated with TBSOTf and 2,4,6-collidine resulting in sphingosine **13**. DMT removal (TFA, addition of dodecanethiol as scavenger)[9], subsequent triflation (Tf_2O, pyridine) followed by *N*-alkylation with ethyleneimine[10] yielded aziridine **15**. Azide reduction, *N*-acylation and TBS removal should yield aziridine **18** as a potential alternative sphingosine-derived ABP.

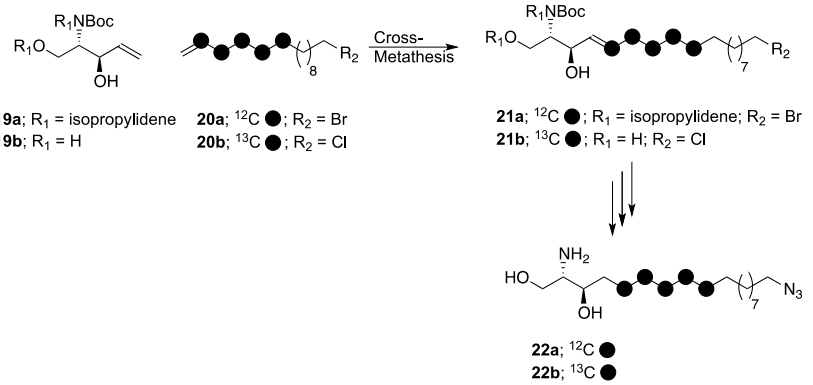
Scheme 7.5 Synthesis towards 1-aziridine ceramide **18.**



Reagents and conditions: (a) TBSOTf, 2,4,6-collidine, DCM 0 °C, 20 h, 65%; (b) dodecanethiol, TFA, DCM, 0 °C, 80%; (c) (i) Tf_2O, pyridine, DCM, 0 °C, 1 h; (ii) ethyleneimine, DCM, r.t., 20 h, 45%.

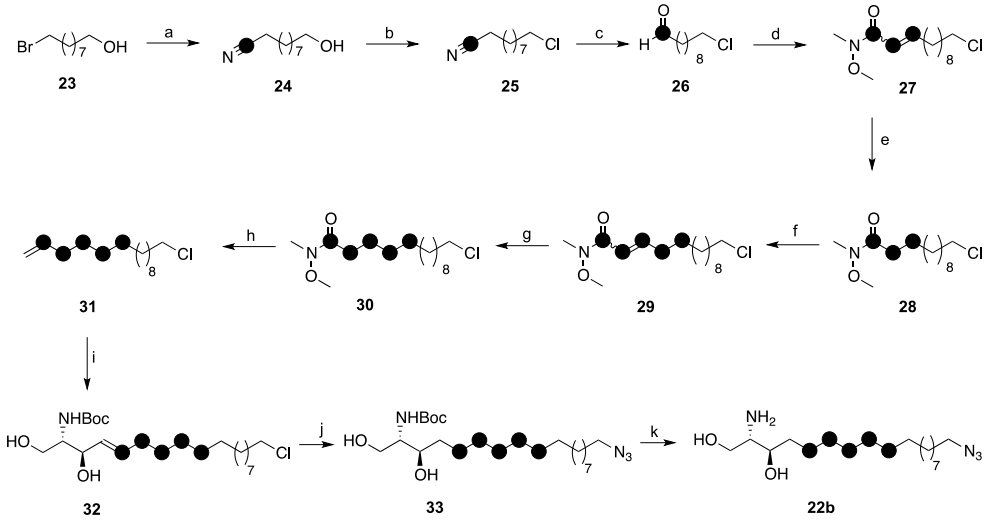
APBs have been proven to be excellent tools to study sphingolipid metabolic enzymes, but in order to get insight in sphingolipid pools as a function of these enzymes another chemical biology approach may be more suited. In a recent article by Delgado and co-workers[11], the sphingolipidome was studied by exposing cells to azide-modified sphinganine **22a** (Scheme 7.6). All sphingolipids are derived from sphinganine and therefore treatment of cells with azido-sphinganine should result in azide tagging of the complete sphingolipidome. The azide can, following lysis of the tissue culture and isolation of the lipid fraction, be addressed as a ligation handle for introduction of, for instance, a fluorophore. Combining the strategy of Delgado and co-workers with the stable-isotope-encoding strategy presented in this Thesis would lead to $^{13}C_5$-labeled azidosphinganine **22b** that, together with its non-isotopically enriched counterpart **22a** may be used for quantitative, chemical metabolomics studies.[12] Cross metathesis of either aminodiol **19a** or **19b** with halogenated alkene **20a**/**b** yielded sphingosine derivatives **21** and **21b**, respectively. Palladium-catalyzed hydrogenation of the double bond, followed by substitution of the halogen for azide and final global deprotection yielded the isotope-code pair of azidosphingosines **22a** and **22b** ready for use in such quantitative chemical lipidomics studies.

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



The synthesis of $^{13}C_5$-azido-sphingosine **22b** was accomplished following the strategy as detailed in Chapter 2[12] and is outlined in Scheme 7.7.

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



Reagents and conditions: (a) K^{13}CN, EtOH/H_2O, 80 °C, 20 h, 99%; (b) NCS, PPh_3, THF, 20 h, 96%; (c) (i) DIBAL-H, THF, -78 °C, 1.5 h; (ii) 1 M HCl (aq), -78 °C, 30 min, 84%; (d) (i) $^{13}C_2$-HWE reagent, *n*-BuLi, 0 °C, 10 min; (ii) **26**, THF, 0 °C to r.t., 20 h, 89%; (e) Pt/C, H_2(g), EtOAc, r.t., 20 h, 93%; (f) (i) DIBAL-H, THF, -78 °C, 1.5 h; (ii) sat. Rochelle salt (aq), -78 °C to r.t.; (iii) $^{13}C_2$-HWE reagent, BuLi, 0 °C, 10 min; (iv) $^{13}C_3$-aldehyde, THF, 0 °C to r.t., 20 h, 94%; (g) (i) DIBAL-H, THF, -78 °C, 1.5 h.; (ii) sat. Rochelle salt (aq), -78 °C to r.t.; (iii) PPh_3MeBr, NaH, THF, 80 °C, 3 h; (iv) $^{13}C_5$-aldehyde, THF, 0 °C to r.t., 20 h, 82%; (h) Grubbs 2nd cat., AcOH, DCM, 40 °C, 2 days, 86%; (i) PtO_2, H_2 (g), EtOAc, 20 h, 86%; (j) NaN_3, NaI, DMF, 55 °C, 20 h, 99%; (k) TFA, H_2O, 0 °C, 30 min, 71%.

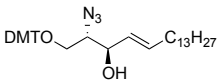
The isotope-encoded sphingolipids described in this thesis have been used for studying the metabolism of sphingolipids in tissue from healthy individuals as well as from patients suffering from various sphingolipidoses.[14-19] Amongst others, the characteristically high

levels of lyso-GB3[14] and glucosylsphingosine[15] as secondary storage lipids in Fabry and Gaucher patients, respectively, can be quantified using the corresponding $^{13}C_5$-enriched sphingolipids as internal standard. By creating a comprehensive panel of stable isotope-enriched sphingolipids, and by developing the chemistry that allows expansion to sphingolipids not yet synthesized in the context of this Thesis, it is expected that the chemical toolset developed will find broader use in metabolomics studies, in relation to lysosomal storage disorders and perhaps as well in other human pathologies.

7.1 Experimental section

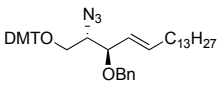
General Remarks: [$^{13}C_2$]-acetic acid (99.95% isotopically pure, product code CLM-105), potassium [^{13}C]-cyanide (99% isotopically pure, product code CLM-297), and [1,2,3-$^{13}C_3$]-myristic acid (99% isotopically pure, product code CLM-3665) was purchased from Cambridge Isotope Laboratories, Inc., and was used as received. Commercially available reagents and solvents (Acros, Fluka, or Merck) were used as received, unless otherwise stated. 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 aluminum 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 phosphormolybdic acid in EtOH (150 g/L), followed by charring (ca. 150 °C). IR spectra were recorded with a Shimadzu FTIR-8300 instrument and are reported in cm^{-1}. Optical rotations were measured with a Propol automatic polarimeter (sodium D-line, λ = 589 nm). ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV 400 MHz spectrometer at 400.2 (^{1}H) and 100.6 (^{13}C) MHz, or with a Bruker AV 600 MHz spectrometer at 600.0 (^{1}H) 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.

(2*S*, 3*S*, 4E)-2-Azido-1-(DMT)octadec-4-ene-3-ol (2). Azidosphingosine **1** (5.2 g, 16.0 mmol, 1.0 eq) was dissolved in dry DCM (80 mL). The solution was cooled to 0 °C followed by addition of 2,4,6-collidine (4.7 mL, 35.2 mmol, 2.2. eq) and DMT-Cl (5.9 g, 17.6 mmol, 1.1 eq) and the reaction was stirred for 2 hours allowing to reach room temperature. The reaction was washed with sat. $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous layers were extracted with DCM (50 mL) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (5% acetone, 1% Et_3N in pentane) giving **2** as a colorless oil (9.3 g, 14.7 mmol, 92%). R_f = 0.23 (5% Acetone in Pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, 2 H, J = 7.6 Hz, H_{arom}), 7.32-7.19 (m, 7 H, H_{arom}), 6.84 (d, 1 H, J = 8.8 Hz, H_{arom}), 5.66 (dt, 1 H, J = 15.2, 6.8 Hz, H-5), 5.33 (dd, 1 H, J = 15.2, 7.2 Hz H-4), 4.19 (m, 1 H, H-3), 3.79 (s, 6 H, OMe_{DMT}), 3.52 (m, 1 H, H-2), 3.29 (d, 2 H, J = 5.6 Hz, H-1), 2.71 (bs, 1

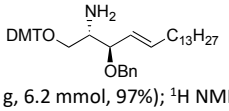


H, OH), 1.96 (q, 2 H, *J* = 6.4 Hz, H-6), 1.30-1.12 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.66, 144.55, 135.76, 135.73 (4x $C_{q\text{-}DMT}$), 135.45 (C-5), 130.11, 130.01, 128.57, 128.17, 128.05, 127.78 (CH_{arom}), 127.03 (C-4), 113.31 ($CH_{arom\text{-}DMT}$), 86.97 ($C_{q\text{-}DMT}$), 73.25 (C-3), 65.89 (C-4), 63.38 (C-1), 55.33 (OMe_{DMT}), 32.38 (C-6), 32.06, 29.82, 29.79, 29.74, 29.59, 29.50, 29.31, 29.04, 22.83 (11x CH_2 C-7 to C-17), 14.27 (C-18); IR (neat) 3415, 2924, 2853, 2101, 1732, 1453, 1270, 1069 cm^{-1}; HRMS calculated for $[C_{39}H_{53}N_3O_4 + H]^+$: 628.4116, found 628.4119.

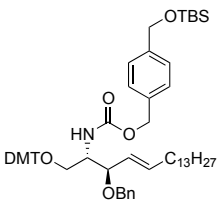
(2*S*, 3*S*, 4E)-2-Azido-1-(DMT)octadec-4-ene-3-*O*-benzyl-ol (3). DMT-protected sphingosine **2** (4.7 g, 7.5 mmol, 1.0 eq) was dissolved in dry DMF (40 mL) under an atmosphere of argon. The solution was cooled to 0 °C and NaH (0.45 g, 11.3 mmol, 1.5 eq) was added and stirred for 30 minutes. To the reaction mixture was added benzyl bromide (1.4 mmol, 12.0 mmol, 1.6 eq) and was left to stir overnight. The reaction mixture was quenched with MeOH (1 mL) and diluted with diethyl ether. The mixture was washed with water and brine. The water layers were extracted with diethyl ether and the combined organic layers were extracted with diethyl ether, dried ($MgSO_4$),filtered and concentrated *in vacuo.* The crude product was purified by silica column chromatography (pentane to 2% EtOAc in Pentane) giving a colorless oil giving a colorless oil (4.2 g, 6.3 mmol, 88%). R_f = 0.4 (2% EtOAc in Pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, 2 H, *J* = 7.6 Hz, $H_{arom\text{-}DMT}$), 7.31-7.24 (m, 12H, H_{arom}), 6.80 (d, 4 H, *J* = 8.8 Hz, H_{arom}), 5.65 (dt, 1 H, *J* = 15.6, 8.8 Hz, H-5), 5.33 (dd, 1 H, *J* = 15.6, 8.8 Hz, H-4), 4.55 (d, 1 H, *J* = 12.0 Hz, $CH_{2a\text{-}Bn}$), 4.27 (d, 1 H, *J* = 12.0 Hz, $CH_{2b\text{-}Bn}$), 3.88 (m, 1 H, H-3), 3.77 (s, 6 H, OMe_{DMT}), 3.57 (m, 1 H, H-2), 3.25 (m, 2 H, H-1), 2.03 (q, 2 H, *J* = 6.8 Hz, H-6), 1.34-1.25 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H *J* = 7.2 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.59, 144.85 (2x $C_{q\text{-}DMT}$), 138.29 ($C_{q\text{-}Bn}$), 137.95 (C-5), 136.05, 136.02 ($C_{q\text{-}DMT}$), 130.19, 130.16, 128.40, 128.27, 127.94, 127.65, 127.56, 126.89 (CH_{arom}), 126.15 (C-4), 113.23 ($CH_{arom\text{-}DMT}$), 86.57 ($C_{q\text{-}DMT}$), 79.53 (C-3), 69.98 ($CH_{2\text{-}Bn}$), 65.17 (C-2), 63.14 (C-1), 55.31 (OMe_{DMT}), 32.47 (C-6), 32.06, 29.83, 29.80, 29.78, 29.60, 29.50, 29.31, 29.16, 22.83 (11x CH_2 C-7 to C-17), 14.26 (C-18); HRMS calculated for $[C_{46}H_{59}N_3O_4 + H]^+$: 718.4586, found 718.4591.



(2*S*, 3*S*, 4E)-2-amino-1-(DMT)octadec-4-ene-3-*O*-benzyl-ol (4). The DMT-Bn-protected sphingosine **3** (4.6 g, 6.4 mmol, 1.0 eq) was dissolved in THF:H_2O (95:5, 40 mL). To the solution was added PMe_3 (1 M in THF, 13 mL, 13 mmol, 2.0 eq) and left to stir overnight. The reaction mixture was concentrated *in vacuo* giving crude sphingosine **4** as a colorless oil (4.29 g, 6.2 mmol, 97%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.16 (m, 14 H, H_{arom}), 6.79 (m, 4 H, $H_{arom\text{-}DMT}$), 5.67 (dt, 1 H, *J* = 15.6, 8.8 Hz, H-5), 5.31 (dd, 1 H, *J* = 15.2, 8.8 Hz, H-4), 4.54 (d, 1 H, *J* = 12.0 Hz, $CH_{2a\text{-}Bn}$), 4.26 (d, 1 H, *J* = 12.0 Hz, $CH_{2b\text{-}Bn}$), 3.78 (m, 1 H, H-3), 3.75 (s, 6 H, OMe_{DMT}), 3.20 (m, 2 H, H-1), 3.09 (m, 1 H, H-2), 2.03 (m, 2 H, H-6), 1.37-1.26 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.47, 145.29 (2x $C_{q\text{-}DMT}$), 138.83 ($C_{q\text{-}Bn}$), 137.29 (C-5), 136.43 ($C_{q\text{-}DMT}$) 130.22, 130.18, 129.25, 128.54, 128.50, 128.42, 128.31, 128.05, 127.87, 127.82, 127.73, 127.38, 127.21, 127.10, 127.03, 126.73 (CH_{arom}), 126.00 (C-4)m 113.11 ($CH_{arom\text{-}DMT}$), 85.92 ($C_{q\text{-}DMT}$), 81.87 (C-3), 69.97 ($CH_{2\text{-}Bn}$), 64.94 (C-1), 55.24 (OMe_{DMT}), 54.97 (C-2), 32.52 (C-6), 32.04, 30.43, 29.81, 29.77, 29.59, 29.47, 29.35, 22.81 (CH_2 C-7 to C-17), 14.24 (C-18); HMRS calculated for $[C_{46}H_{61}NO_4 + H]^+$: 692.4681, found 692.4679.

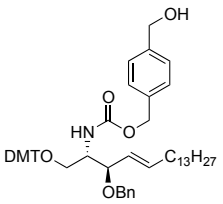


(2*S*, 3*S*, 4E)-2-*N*-(4-(OTBS)methyl)benzylcarbamate-1-(ODMT)-octadec-4-ene-3-*O*-benzyl-ol (6). Sphingosine **4** (4.1 g, 6.0 mmol, 1.0 eq) was dissolved in dry DMF (30 mL) under protected atmosphere. To the solution was DIPEA (1.35 mL, 7.8 mmol, 1.3 eq) added followed by addition of nitrophenol linker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



7.15 (m, 16 H, H_{arom}), 6.77-6.73 (m, 4 H, $H_{arom\text{-}DMT}$), 5.69 (dt, 1 H, *J* = 15.2, 8.0 Hz, H-5), 5.32 (dd, 1 H, *J* = 15.2, 8.0 Hz, H-4), 5.05 (s, 2 H, $CH_{2\text{-}Linker}$), 5.02 (d, 1 H, *J* = 8.0 Hz, NH), 4.73 (s, 2 H, $CH_{2\text{-}Linker}$), 4.56 (d, 1 H, *J* = 12.0 Hz, $CH_{2a\text{-}Bn}$), 4.28 (d, 1 H, *J* = 12.0 Hz, $CH_{2b\text{-}Bn}$), 4.03-3.94 (m, 2 H, H-2 and H-3), 3.73 (s, 6 H, OMe_{DMT}), 3.47 (m, 1 H, H-1_a), 3.19 (dd, 1 H, *J* = 9.2, 3.6 Hz, H-1_b), 2.02 (m, 2 H, H-6), 1.36-1.23 (m, 22 H, H-7 to H-17), 0.94 (s, 9 H, tBu_{TBS}), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18), 0.10 (s, 6 H, Me_{TBS}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.53 ($C_{q\text{-}DMT}$), 156.06 ($C{=}O_{linker}$), 145.02, 141.48, 138.55 (3x $C_{q\text{-}arom}$), 137.32 (C-5), 136.10, 135.31 (2x $C_{q\text{-}arom}$), 130.23, 130.12, 129.24, 128.35, 128.26, 128.09, 127.87, 127.69, 127.46, 127.32, 127.05, 126.81 (CH_{arom}), 126.23(C-4), 126.10 (CH_{arom}), 133.10 ($CH_{arom\text{-}DMT}$), 86.08 ($C_{q\text{-}DMT}$), 80.17 (C-3), 70.29 ($CH_{2\text{-}Bn}$), 66.59 ($CH_{2\text{-}Linker}$), 64.79 ($CH_{2\text{-}Linker}$), 61.87 (C-1), 55.24 (OMe_{DMT}), 54.43 (C-2), 32.42 (C-6), 32.04, 29.83, 29.78, 29.64, 29.48, 29.37, 29.30 (CH_2 C-7 to C-17), 26.06 ($CH_{3\text{-}tBu\text{-}TBS}$), 22.81 ($CH_2$ C-7 to C-17), 18.51 ($C_{q\text{-}tBu\text{-}TBS}$), 14.25 (C-18), -5.15 ($Me_{TBS}$); HRMS calculated for $[C_{61}H_{83}NO_7Si + H]^+$: 970.6019, found 970.6023.

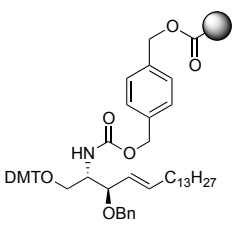
(2*S*, 3*S*, 4E)-2-*N*-(4-(hydroxy)methyl)benzylcarbamate-1-ol-octadec-4-ene-3-*O*-benzyl-ol (7). Protected sphingosine-linker **6** (1.94 g, 2.0 mmol, 1.0 eq) was dissolved in THF (10 mL) and cooled to 0 °C. To the solution was added TBAF (1 M in THF, 3.0 mL, 3.0 mmol, 1.5 eq) and the reaction was stirred for 2 hours allowing to reach room temperature. The mixture was diluted with EtOAc and washed with water and brine. The water layers were extracted with EtOAc and combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (5-10% EtOAc in pentane) giving a colorless oil (1.68 g, 1.96 mmol, 98 %).



R_f = 0.30 (10% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.17 (m, 18 H, H_{arom}), 6.72 (d, *J* = 8.8 Hz, $H_{arom\text{-}DMT}$), 5.69 (dt, 1 H, *J* = 11.6, 8.4 Hz, H-5), 5.26 (dd, 1 H, *J* = 11.6, 8.4 Hz, H-4), 5.12-4.97 (m, 3 H, $CH_{2\text{-}Linker}$ and NH), 4.66 (s, 2 H, $CH_{2\text{-}linker}$), 4.56 (d, 1 H, *J* = 11.6 Hz, $CH_{2a\text{-}Bn}$), 4.27 (d, 1 H, *J* = 11.6 Hz, $CH_{2\text{-}Bn}$), 4.01-3.95 (m, 2 H, H-2 and H-3), 3.74 (d, 6 H, *J* = 3.6 Hz, OMe_{DMT}), 3.45 (m, 1 H, H-1_a), 3.20 (m, 1 H, H-1_b), 2.02 (m, 2 H, H-6), 1.42-1.26 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.44 ($C_{q\text{-}DMT}$), 156.00 ($C{=}O_{linker}$), 145.01, 140.97, 138.49 ($C_{q\text{-}arom}$), 137.06 (C-5), 136.15 ($C_{q\text{-}arom}$), 130.16, 129.24, 128.82, 128.62, 128.41, 128.35, 128.26, 127.93, 127.86, 127.70, 127.48, 127.27, 127.21, 127.18 (CH_{arom} and C-4), 113.14 ($CH_{arom\text{-}DMT}$), 86.06 ($C_{q\text{-}DMT}$), 80.10 (C-3), 70.30 ($CH_{2\text{-}Bn}$), 66.39 ($CH_{2\text{-}linker}$), 65.04 ($CH_{2\text{-}linker}$), 61.87 (C-1), 55.26 (OMe_{DMT}), 54.46 (C-2), 32.43, 32.06, 29.82, 29.78, 29.63, 29.57, 29.47, 29.36, 29.29, 29.17, 22.80 (C-6 to C-17), 14.25 (C-18); HRMS calculated for $[C_{55}H_{69}NO_7 + H]^+$: 856.5154, found 856.5149.

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

Coupling basic cleavable sphingosine linker to resin (8). The resin was swollen with DCM (5 mL) for 15 minutes, followed by filtration. To the swollen resin DCM (9 mL) was added followed by addition of a solution existing of sphingosine linker **7** (1.4 g, 1.65 mmol, 3.0 eq) and DIC (0.26 mL, 1.65 mmol, 3.0 eq) in DCM (4 mL). Next, DMAP (10 mg, 0.05 mmol, 0.1 eq) was added and the reaction was left to shake overnight. The reaction was quenched by addition of MeOH (0.15 mL) and left to shake for 1 hour. The resin was filtered and washed three times with DCM, followed by swelling/shrinking DCM/hexane procedure. Finally, the resin was washed with three times with DCM and then dried.



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

(2*S*, 3*S*, 4E)-2-Amino-1-(DMT)octadec-4-ene-3-ol (10). Protected azidosphingosine **2** (1.0 g, 1.6 mmol, 1 eq) was dissolved in EtOH (8 mL). The solution was purged with argon before the addition of Lindlar catalyst (5% wt. Pd on $CaCO_3$, poisoned with lead). The reaction mixture was purged with H_2 (g) for 15 minutes. The reaction was stirred overnight under an atmosphere of hydrogen gas. The mixture was filtered over Celite and concentrated *in* vacuo. The crude product was purified by silica column chromatography (2% MeOH, 1% Et_3N in DCM) giving a colorless oil (0.54 g, 0.90 mmol, 54%). R_f = 0.5 (5% MeOH in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, 2 H, *J* = 7.6 Hz, $H_{arom\text{-}DMT}$), 7.31-7.18 (m, 7 H, $H_{arom\text{-}DMT}$), 6.82 (d, 4 H, *J* = 7.2 Hz, $H_{arom\text{-}DMT}$), 5.64 (dt, 1 H, *J* = 15.2, 7.6 Hz, H-5), 5.26 (dd, 1 H, *J* = 15.2, 6.8 Hz, H-4), 4.12 (t, 1 H, *J* = 6.0 Hz, H-3), 3.78 (s, 6 H, OMe_{DMT}), 3.18 (d, 2 H, *J* = 5.6 Hz, H-1), 3.02 (m, 1 H, H-2), 2.42 (bs, 2 H, NH_2), 1.94 (m, 2 H, H-6), 1.34-1.23 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.58, 144.90, 136.13, 136.09 ($C_{q\text{-}DMT}$), 134.16 (C-5), 130.11, 129.24, 128.79, 128.20, 128.17, 127.88, 127.74 ($CH_{arom\text{-}DMT}$), 126.91 (C-4), 113.26 ($CH_{arom\text{-}DMT}$), 86.43 ($C_{q\text{-}DMT}$), 74.84 (C-3), 65.81 (C-1), 55.30 (OMe_{DMT}), 46.29 (C-2), 32.04 (C-6), 29.82, 29.78, 29.65, 29.61, 29.48, 29.37, 29.27, 22.81 (CH_2 C-7 to C-17), 14.24 (C-18); IR (neat) 3330, 2914, 2849, 1661, 1469, 1198 cm^{-1}; HRMS calculated for $[C_{39}H_{55}NO_4 + H]^+$: 602.4211, found 602.4223.

(2*S*, 3*S*, 4E)-2-*N*-(5-hydroxy-2-nitrobenzyl)-1-(DMT)octadec-4-ene-3-ol (11). The DMT protected sphingosine **10** (0.5 g, 0.83 mmol, 1 eq) was dissolved in wet THF (3 mL). 5-hydroxy-2-nitrobenzaldehyde (0.13 g, 0.83 mmol, 1.0 eq) was added and stirred for one hour. To the reaction was added $NaBH_3CN$ (52 mg, 0.83 mmol, 1.0 eq) was added and mixture was stirred overnight at room temperature. The reaction was quenched with $NaHCO_3$ (10 mL) and EtOAc (10 mL). The two layers were separated and the water layer was extracted with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$, filtered and concentrated *in vacuo.* Purification by column chromatography (20% EtOAc in Pentane) giving a yellow oil (0.38 g, 0.5 mmol, 62%) NMR will depend on acidity of the chloroform. R_f = 0.27 (30% EtOAc in Pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, 1 H, *J* = 9.2 Hz, $H_{arom\text{-}nitro\ phenol}$), 7.38 (d, 2 H, *J* = 7.2 Hz, $H_{arom\text{-}DMT}$), 7.29-7.14 (m, 7 H, $H_{arom\text{-}DMT}$), 6.82-6.79 (m, 5 H, 4x $H_{arom\text{-}DMT}$ and $H_{arom\text{-}nitro\ phenol}$), 6.58 (dd, 1 H, *J* = 9.2, 2.0 Hz, $H_{arom\text{-}nitro\ phenol}$), 5.65 (dt, 1 H, *J* = 15.6, 7.6 Hz, H-5), 5.20 (dd, 1 H, *J* = 15.2, 6.0 Hz, H-4), 4.35 (m, 1 H, H-3), 4.06 (d, 1 H, *J* = 14.0 Hz, $CH_{2a\text{-}nitrophenol}$), 3.94 (d, 1 H, *J* = 14.0 Hz, $CH_{2\text{-}nitrophenol}$), 3.73 (s, 6 H, OMe_{DMT}), 3.41 (m, 2 H, H-1), 2.91 (m, 1 H, H-2), 1.91 (m, 2 H, H-6), 1.33-1.22 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.43 ($C_{q\text{-}nitro\ phenol}$), 158.78, 144.35 ($C_{q\text{-}arom\text{-}DMT}$), 139.38 (C-5), 139.09 ($C_{q\text{-}nitro\ phenol}$), 135.41, 135.26 ($C_{q\text{-}DMT}$), 130.15,130.13, 129.26, 128.80, 128.20, 127.24, ($CH_{arom\text{-}DMT}$ and $CH_{nitrophenol}$), 125.34 (C-4), 115.50, 114.38 ($CH_{nitrophenol}$), 133.48 ($CH_{arom\text{-}DMT}$), 78.52 (C-3), 62.48 (C-2), 59.59 (C-1), 55.34 (OMe_{DMT}), 46.21 ($CH_{2\text{-}nitrophenol}$), 32.48, 32.05, 29.83, 29.80, 29.62, 29.50, 29.41, 29.09, 22.82 (C-6 to C-17), 14.29 (C-18); HRMS calculated for $[C_{46}H_{60}N_2O_7 + H]^+$: 753.4481, found 753.4494.

(2*S*, 3*S*, 4E)-2,3-*O,N*-carbamate-2-*N*-(5-hydroxy-2-nitrobenzyl)-1-(DMT)octadec-4-ene (12). The nitro phenol sphingosine **11** (0.18 g, 0.4 mmol, 1.0 eq) was dissolved in dry DCM. To the solution was added carbonyl-diimidazole (1.23 g, 7.6 mmol, 2 eq) and was stirred overnight at room temperature. The mixture was concentrated and purified by silica column chromatography (10% EtOAc in Pentane) giving a yellow oil (0.17g 0.36 mmol, 90%). R_f = 0.45 (20% EtOAc in Pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, 1 H, *J* = 9.2 Hz,

$H_{arom\text{-}nitro\ phenol}$), 7.40-7.21 (m, 9 H, $H_{arom\text{-}DMT}$), 6.88-6.81 (m, 5 H, 4x $H_{arom\text{-}DMT}$ and $H_{arom\text{-}nitro\ phenol}$), 6.70 (dd, 1 H, *J* = 8.8, 2.4 Hz, $H_{arom\text{-}nitro\ phenol}$), 5.81 (dt, 1 H, *J* = 15.6, 8.4 Hz, H-5), 5.52 (dd, 1 H, *J* = 15.2, 8.4 Hz, H-4), 5.21 (t, 1 H, *J* = 8.4 Hz, H-3), 5.02 (d, 1 H, *J* = 18.4 Hz, $CH_{2a\text{-}nitrophenol}$), 4.57 (d, 1 H, *J* = 17.6 Hz, $CH_{2\text{-}nitrophenol}$), 3.81-3.74 (m, 7 H, OMe_{DMT} and H-2), 3.55 (dd, 1 H, *J* = 10.8, 3.2 Hz, H-1_a), 3.13 (dd, 1 H, *J* = 10.8, 2.4 Hz, H-1_b), 1.88 (m, 2 H, H-6), 1.34-1.19 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.09 ($C_{q\text{-}nitrophenol}$), 158.80, 144.00 ($C_{q\text{-}nitrophenol}$), 140.67 (C-5), 135.21, 135.06 ($C_{q\text{-}DMT}$), 130.15, 130.03, 129.75, 129.34, 128.80, 128.20, 127.24 ($CH_{arom\text{-}DMT}$ and $CH_{arom\text{-}nitro\ phenol}$), 122.25 (C-4), 115.56 114.31 ($CH_{arom\text{-}nirophenol}$), 113.48, 113.42 ($CH_{arom\text{-}DMT}$), 79.78 (C-3), 60.88 (C-2), 59.69 (C-1), 55.37 (OMe_{DMT}), 43.96 ($CH_{2\text{-}nitrophenol}$), 32.46, 32.09, 29.89, 29.70, 29.53, 29.47, 29.03, 22.85 (C-6 to C-17), 14.29 (C-18); IR (neat); HRMS calculated for $[C_{47}H_{58}N_2O_8 + H]^+$: 779.4273, found 779.4278.

(2*S*, 3*S*, 4E)-2-Azido-1-(DMT)octadec-4-ene-3-*O*-TBS. (13). 1-DMT-sphingosine **2** (1.25g, 2 mmol, 1.0 eq) was dissolved in dry DCM (10 mL) under protected atmosphere and cooled to 0 °C. 2,4,6-Collidine (0.65 mL, 5 mmol, 2.5 eq) was added to the solution followed by addition of TBSOTf (0.57 mL, 2.5 mmol, 1.25 eq). The reaction was stirred overnight at 4 °C. The reaction was diluted with DCM and washed with sat. aq. $NaHCO_3$ and brine. The water layers were extracted with DCM and the combined organic layers were dried ($MgSO_4$), filtered and concentrated *in* vacuo. The product was purified by silica column chromatography (1% to 5% Acetone in Pentane) giving a colorless oil (0.93 g, 1.3 mmol, 65%). R_f = 0.6 (5% Aceton in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, 2 H, *J* = 7.6 Hz, $H_{arom\text{-}DMT}$), 7.33-7.29 (m, 12 H, H_{arom}), 6.81 (dd, 4 H, *J* = 8.8, 1.6 Hz, H_{arom}), 5.52 (dt, 1 H, *J* = 15.2, 8.8 Hz, H-5), 5.32 (dd, 1 H, *J* = 15.2, 7.2 Hz, H-4), 4.07 (m, 1 H, H-3), 3.78 (s, 6 H, OMe_{DMT}), 3.54 (m, 1 H, H-2), 3.15 (m, 2 H, H-1), 2.03 (m, 2 H, H-6), 1.34-1.24 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H *J* = 7.2 Hz, H-18), 0.78 (s, 9 H, TBS_{tBu}), -0.05 (s, 3 H, TBS_{Me}), -0.07 (s, 3 H, TBS_{Me}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.60, 144.89, 136.19, 136.06 (2x $C_{q\text{-}DMT}$), 134.34 (C-5), 130.16, 130.10, 129.00, 128.26, 127.93 127.73 ($CH_{arom\text{-}DMT}$), 126.80 (C-4), 113.23 ($CH_{arom\text{-}DMT}$), 86.57 ($C_{q\text{-}DMT}$), 74.52 (C-3), 67.44 (C-2), 63.45 (C-1), 55.31 (OMe_{DMT}), 32.31, 32.06, 29.83, 29.81, 29.76, 29.60, 29.49, 29.31, 29.15, (11x CH_2 C-6 to C-17), 25.83 (TBS_{tBu}), 22.82 (CH_2 C-6 to C17), 18.09 ($TBS_{cq\text{-}tBu}$) 14.25 (C-18), -3.98, -4.89 (2x TBS_{Me}).

(2*S*, 3*S*, 4E)-2-Azido-1-octadec-4-ene-3-*O*-TBS. (14). Silylated sphingosine **13** (0.9 g, 1.22 mmol, 1.0 eq) was dissolved in dry DCM (12 mL) under protected atmosphere and dodecanethiol (0.6 mL, 2.5 mmol, 2.0 eq) was added to solution. The solution was cooled to 0 °C, followed by addition of trifluoracetic acid (72 µL, 0.96 mmol, 0.8 eq) and the reaction was left to stir for 2 hours. The reaction was diluted with DCM and was washed with sat. aq. $NaHCO_3$ and brine. The water layers were extracted with DCM and the combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo.* The product was purified by silica column chromatography giving a colorless oil (0.42 g, 0.96 mmol, 80%). R_f = 0.15 (5% Aceton in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (dt, 1 H, *J* = 15.6, 7.2 Hz, H-5), 5.40 (dd, 1 H, *J* = 15.6, 7.2 Hz, H-4), 4.17 (m, 1 H, H-3), 3.66-3.62 (m, 2 H, H-1), 3.36 (m, 1 H, H-2), 2.20 (bs, 1 H, OH), 2.00 (m, 2 H, H-6), 1.34 (m, 2 H, H-7), 1.30-1.20 (m, 20 H, H-8 to H-17), 0.89-0.81 (m, 12 H, H-18 and TBS_{tBu}), 0.04 (s, 3 H, TBS_{Me}), -0.01 (s, 3 H, TBS_{Me}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.92 (C-5), 129.14 (C-4), 75.39 (C-3), 67.83 (C-2), 62.37 (C-1), 32.38, 32.27, 32.07, 29.84, 29.82, 29.75, 29.60, 29.33, 29.10, 28.80 (11x CH_2 C-6 to C-17), 25.87 (TBS_{tBu}), 22.82 (CH_2 C-6 to C17), 18.16 ($TBS_{cq\text{-}tBu}$) 14.25 (C-18), -3.96, -4.88 (2x TBS_{Me}).

(2*S*, 3*S*, 4E)-2-Azido-1-(ethyleenime)-octadec-4-ene-3-*O*-TBS. (15). Sphingosine **14** (0.4 g, 0.91 mmol, 1.0 eq) was dissolved in dry DCM (5 mL) under protected atmosphere and cooled to 0 °C. Pyridine (88 µL, 1.1 mmol, 1.2 eq) was added followed by addition of Tf_2O (0.18 mL, 1.1 mmol, 1.2 eq). The mixture was stirred for 1 hour following dilution with DCM (10 mL). The reaction was washed with water and brine. The water layers were extracted with DCM and the combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo* giving the crude triflate, which directly used without any further purification in the next step. The crude triflated sphingosine was dissolved in dry DCM (5 mL) under protected atmosphere and cooled to -20 °C. DIPEA (0.19 mL, 1.1 mmol, 1.2

eq) was added followed by addition of dry ethyleneimine (1 mL 20 mmol, 20 eq), and was left stirring for 3 hours. The reaction mixture was quenched with MeOH (0.1 mL) and washed with water and brine. The water layers were extracted with DCM and combined organic layers were dried ($MgSO_4$) filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (pentane to 2% acetone in pentane) giving a colorless oil (0.18 g, 0.40 mmol 45%). $[\alpha]^{20}_D$: -26 (C= 0.5, $CHCl_3$); R_f = 0.72 (5% acetone in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dt, 1 H, *J* = 15.2, 7.6 Hz, H-5), 5.42 (dd, 1 H, *J* = 15.6, 7.2 Hz, H-4), 4.19 (m, 1 H, H-3), 3.54 (m, 1 H, H2), 2.36 (dd, 1 H, *J* = 12.8, 4.0 Hz, H-1_a), 2.10 (dd, 1 H, *J* = 12.4, 8.4 Hz, H-1_b), 2.03 (q, 2 H, *J* = 7.2 Hz, H-6), 1.84 (dd, 1 H, *J* = 6.0, 4.0 Hz, $H_{aziridine}$), 1.75 (dd, 1 H, *J* = 6.0, 4.0 Hz, $H_{aziridine}$), 1.40-1.24 (m, 23 H, H-7 to H-17 and $H_{aziridine}$), 1.10 (dd, 1 H, *J* = 6.0, 4.0 Hz, $H_{aziridine}$), 0.91-0.86 (m, 12 H, H-18 and TBS_{tBu}), 0.06 (s, 3 H, TBS_{Me}), 0.03 (s, 3 H, TBS_{Me}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.56 (C-5), 128.86 (C-4), 75.53 (C-3), 67.92 (C-2), 61.23 (C-1), 32.36, 32.05, 29.81, 29.78, 29.76, 29.68, 29.60, 29.49, 29.15, 28.98, 28.23, 27.03, 26.01 (11x CH_2 C-6 to C-17 and 2x CH_2-aziridine), 25.90 (TBS_{tBu}), 18.21 ($TBS_{Cq\text{-}tBu}$), 14.25 (C-18), -3.98, -4.80 (2x TBS_{Me}).

[1-$^{13}C_1$]-10-Hydroxydecanitrile (24). 9-Bromo-1-nonanol **23** (8.87 g, 39.8 mmol, 1.05 eq) was dissolved in EtOH/H_2O (9:1, 17 mL), and K^{13}CN (2.5 g, 37.9 mmol) was added. The reaction mixture was heated at 80 °C overnight. The reaction was then diluted with ether and was washed 2x with water and 1x with brine. The water layers were extracted with ether and the combined organic layer were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by column chromatography (20% EtOAc in petroleum ether) giving colorless oil (6.43 g, 37.8 mmol, 99%). R_f = 0.51 (1:1 EtOAc: petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (t, 2 H, *J* = 6.8 Hz, H-10), 2.34 (dt, 2 H, *J* = 9.6, 7.2 Hz, H-2), 1.69-1.62 (m, 3 H, H-9 and OH), 1.55 (m, 2 H, H-3), 1.44 (m, 2 H, H-8), 1.39-1.28 (m, 8 H, H-4 to H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 119.9 (CN), 63.8 (C-OH), 32.7, 29.3 (d, *J* =1.5 Hz), 28.71, 28.65 (d, *J* = 3.3 Hz) 25.7, 25.35 (d, *J* = 2.7 Hz), 17.1 (d, *J* = 56.6 Hz, C-2). IR (neat): 3294, 2926, 2854, 2193, 1463, 1425, 1055 cm^{-1}. HRMS calculated for $[C_9{}^{13}CH_{19}NO + H]^+$: 171.1466, found 171.1470.

[1-$^{13}C_1$]-10-Chloro-decanitrile (25). [1-$^{13}C_1$]-10-Hydroxydecanitrile **24** (6.21 g, 36.5 mmol) was dissolved in THF (240 mL) to which PPh_3 (10.5 g, 40.2 mmol, 1.1 eq) and NCS (5.36 g, 40.2 mmol, 1.1 eq) were added. The mixture was stirred overnight at room temperature. After the reaction was finished on TLC the mixture was diluted with EtOAc and 2x washed with water and 1x with brine. The water layers were extracted with EtOAc and the combined organic layers were dried (Na_2SO_4) and concentrated. Petroleum ether was added to residue and the remaining solids were filtered off and the organic solution was concentrated *in vacuo*. The product was purified by column chromatography (3% EtOAc in petroleum ether) giving a colorless oil (6.60 g, 35.0 mmol, 96%). R_f = 0.24 (3% EtOAc in petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 (t, 2 H, *J* = 6.8 Hz, H-10), 2.34 (dt, 2 H, *J* = 9.6, 7.2 Hz, H-2), 1.77 (p, 2 H, *J* = 6.9 Hz, H-9), 1.65 (m, 2 H, H-3), 1.48-1.37 (m, 4 H, H-4 and H-8), 1.34-1.25 (m, 6 H, H-5 to H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 119.7 (CN), 45.0 (C-10), 32.4 (C-9), 29.00, 28.59, 28.52, 28.46 (d, *J* = 3.3 Hz), 26.66 (5x -CH_2-, C-4 to C-8), 25.2 (d, *J* = 2.7 Hz, C-3), 17.0 (d, *J* =55.5 Hz, C-2). IR (neat); 2928, 2857, 2193, 1462, 1427, 1307, 721, 648 cm^{-1}. HRMS calculated for $[C_9{}^{13}CH_{18}NCl + H]^+$: 189.1206, found 189.1208.

[1-$^{13}C_1$]- 10-chlorodecanal (26). [1-$^{13}C_1$]-10-Chloro-decanitrile **25** (2.09 g, 10.66 mmol) was dissolved in dry DCM (40 mL) and was cooled to -78 °C under protected atmosphere. DIBAL-H (1.5 M in toluene, 10.67 mL, 16.0 mmol, 1.5 eq) was slowly added, and stirred for 1.5 h at -78 °C. The reaction mixture was quenched with 1 M HCl (aq) (15 mL) and was stirred for 30 minutes at -78 °C. Then the reaction mixture was warmed to room temperature and was diluted with ether. The mixture was washed 2x with water and 1x with brine. The water layers were extracted with ether and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by column chromatography (3% EtOAc in petroleum ether) giving a colorless oil (1.71 g, 8.95 mmol, 84 %). R_f = 0.25 (3% EtOAc in petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (dt, 1 H, *J* = 169.6, 1.6 Hz, H-1), 3.52 (t, 2 H, *J* = 6.8 Hz, H-10), 2.43 (dq, 2 H, *J* = 7.2, 1.6 Hz, H-2), 1.78 (p, 2 H, *J* = 6.8 Hz, H-9), 1.63 (m, 2 H, H-3), 1.42(m, 2 H, H-8), 1.41-1.27 (m, 8 H, H-4 to H-7). ^{13}C

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

[1,2,3-$^{13}C_3$]-(E)-12-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide and [1,2,3-$^{13}C_3$]-(Z)-12-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide (27). Diethyl ([1,2-$^{13}C_2$]-*N*-methoxy-*N*-methylacetamide)phosphonate (2.46 g, 10.2 mmol, 1.05) was dissolved in dry THF (47 mL) under protected atmosphere. n-BuLi (1.6 M in hexane, 6.37 mL, 10.2 mmol, 1.05 eq) was added at 0 °C and stirred for 10 min at this temperature. [1-$^{13}C_1$]- 10-chlorodecanal **26** (1.86 g, 9.71 mmol) was dissolved in dry THF (10.0 mL) was added to the phosphonate carbanion and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with ether and was washed 2x with water and 1x brine. The water layers were extracted with ether and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by column chromatography (10% EtOAc in petroleum ether) giving a colorless oil (2.41 g, 8.64 mmol, 89%, E:Z = 11:1). R_f (**E**) = 0.12 and R_f (**Z**) = 0.20 (10% EtOAc in petroleum ether). (***E*-isomer**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (dm, 1 H, *J* = 154.0 Hz, H-3), 6.41 (ddd, 1 H, *J* = 160.4, 15.2, 3.6 Hz, H-2), 3.70 (s, 3 H, $CH_{3\text{-}OMe}$), 3.53 (t, 2 H, *J* = 6.4 Hz, H-12), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$), 2.24 (m, 2 H, H-4), 1.76 (p, 2 H, *J* = 6.8 Hz, H-11), 1.52-1.38 (m, 4 H, H-5 and H-10), 1.37-1.20 (m, 8 H, H-6 to H-9). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1 (d, *J* = 67.7 Hz, C-1), 148.0 (d, *J* = 71.7 Hz, C-3), 118.6 (dd, *J* = 71.7, 67.7 Hz, C-2), 61.7 ($CH_{3\text{-}OMe}$), 45.2 (C-12), 32.7 (C-11), 32.6 (m, C-4), 31.8 (m, $CH_{3\text{-}NMe}$), 29.41, 29.35, 29.3 (d, *J* = 3.6 Hz), 28.9, 28.35 (m), 26.9 (6x CH_2 C-5 to C-10). IR (neat) 2926, 2854, 1616, 1581, 1462, 1367, 1177, 983 cm^{-1}. (**Z-isomer**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.24 (dd, 1 H, *J* = 162.0, 10.4 Hz, H-2), 6.11 (dm, 1 H, *J* = 152.4 Hz, H-3), 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.53 (t, 2 H, *J* = 6.8 Hz, H-12), 3.21 (s, 3 H, $CH_{3\text{-}NMe}$), 2.61 (m, 2 H, H-4), 1.76 (p, 2 H, *J* = 7.0 Hz, H-11), 1.49-1.37 (m, 4 H, H-5 and H-10), 1.36-1.22 (m, 8 H, H-6 to H-9). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.6 (d, *J* = 66.7 Hz, C-1), 147.7 (d, *J* = 71.7 Hz, C-3), 117.9 (t, *J* = 69.7 Hz, C-2), 61.5 ($CH_{3\text{-}OMe}$), 45.2 (C-12), 32.63 (C-11), 32.2-31.8 (m, $CH_{3\text{-}NMe}$ and C-4), 29.45, 29.31, 2x 29.4-29.2 (m), 28.9, 26.94 (6x CH_2 C-5 to C-10). IR (neat); 2926, 2855, 1612, 1460, 1332, 1177, 997 cm^{-1}. HRMS calculated for $[C_{11}{}^{13}C_3H_{26}NO_2Cl + H]^+$: 279.1652, found 279.1651.

[1,2,3-$^{13}C_3$]-12-Chloro-*N*-methoxy-*N*-methyl-dodecanamide (28). [1,2,3-$^{13}C_3$]-(E/Z)-12-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide **27** (5.76 g, 20.68 mmol) was dissolved in EtOAc (240 mL). The solution was purged with argon under stirring and a catalytic amount of palladium 10% on charcoal (1.0 g, 1.0 mmol, 0.05 eq) was added. The reaction was stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere overnight. The palladium was removed by filtration over a plug of Celite and then rinsed with EtOAc followed by removal of the solvent under reduced pressure giving the desired product, a colorless oil (5.40 g, 19.3 mmol, 93%). R_f = 0.15 (10% EtOAc in petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.53 (t, 2 H, *J* = 6.8 Hz, H-12), 3.18 (s, 3 H, $CH_{3\text{-}NMe}$), 2.41 (dm, 2 H, *J* = 127.6 Hz, H-2), 1.82-1.72 (m, 2 H, H-11), 1.64 (dm, 2 H, *J* = 127.6 Hz, H-3), 1.54-1.38 (m, 2 H, H-10), 1.37-1.22 (m, 12 H-4 to H-9). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.9 (d, *J* = 51.5 Hz, C-1), 61.3 ($CH_{3\text{-}OMe}$), 45.3 (C-12), 32.64 (C-11), 32.2 (dd, *J* = 51.5, 37.2 Hz, C-2 and $CH_{3\text{-}NMe}$), 29.48 (m, C-4), 28.87, 26.85, 24.64 (d, *J* = 35.1 Hz, C-3), 24.7 (7x CH_2 C-4 to C-10). IR (neat); 2924, 2852, 1626, 1464, 1371 1175, 1001 cm^{-1}. HRMS calculated for $[C_{11}{}^{13}C_3H_{28}NO_2Cl + H]^+$: 281.1808, found 281.1805.

[1,2,3,4,5-$^{13}C_5$]-(E)-14-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide and [1,2,3,4,5-$^{13}C_5$]-(Z)-14-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide (29). [1,2,3-$^{13}C_3$]-12-Chloro-*N*-Methoxy-*N*-methyl-dodecanamide **28** (5.29 g, 18.6 mmol) was dissolved in dry THF (75 mL) and was cooled to -78°C under protected atmosphere. DIBAL-H (1.5 M in toluene, 14.9 mL, 22.4 mmol, 1.2 eq) was added and the mixture was stirred for 1 hour at -78°C. The mixture was quenched with sat. Rochelle salt (aq) (75 mL) at -78°C and was roughly stirred to room temperature. The mixture was diluted with ether and was washed 2x with water and 1 x with brine. The water layers were extracted with ether and the combined organic layers was dried (Na_2SO_4) and concentrated *in vacuo* giving crude [1,2,3- $^{13}C_3$]-12-Chloro-dodecanal as a clear oil which was used without further purification. [1,2-$^{13}C_2$]-

Diethyl (*N*-Methoxy-*N*-methylacetamide)phosphonate (4.33 g, 18.0 mmol, 1.05) was dissolved in dry THF (90 mL) under protected atmosphere. *n*-BuLi (1.6 M in hexane, 11.2 mL, 18.0 mmol, 1.05 eq) was added at 0 °C and stirred for 10 min at this temperature. 12-Chloro-dodecanal (3.80 g, 17.1 mmol) was dissolved in dry THF (18 mL) was added to the phosphonate carbanion and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with ether and was washed 2x with water and 1x with brine. The water layers were extracted with ether and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by column chromatography (10% EtOAc in petroleum ether) giving a colorless oil (5.41 g, 17.5 mmol, 94%, E:Z = 10.5: 1). R_f (**E**) = 0.2 and R_f (**Z**) =0.31 (10 % EtOAc in petroleum ether). (**E-isomer**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (dm, 1 H, *J* = 156.0 Hz, H-3), 6.38 (ddm, 1 H, *J* = 160.4, 15.6 Hz, H-2), 3.70 (s, 3 H, $CH_{3\text{-}OMe}$), 3.53 (t, 2 H, *J* = 6.8 Hz, H-14), 3.24 (s, 3 H, $CH_{3\text{-}NMe}$), 2.21 (dm, 2 H, *J* = 127.2 Hz, H-4), 1.76 (p, 2 H *J* = 7.0 Hz, H-13), 1.68-1.20 (m, H 16, H-5 to H-12). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2 (dd, *J* = 67.1, 5.7 Hz, C-1), 148.1 (dd, *J* = 71.7, 41.2 Hz, C-3), 118.7 (ddd, *J* = 71.7, 67.1, 4.6 Hz, C-2), 61.8 ($CH_{3\text{-}OMe}$), 45.3 (C-14), 32.5 (C-13), 32.4 (dddd, *J* = 41.2, 33.8, 5.7, 1.3 Hz, C-4), 32.2 ($CH_{3\text{-}NMe}$), 29.6-29.0 (6x CH_2 C-6 to C-11), 28.6 (dd, *J* = 36.6, 33.8 Hz, C-5), 27.0 (C-12). IR (neat); 2922, 2852, 1616, 1581, 1462, 1368, 1177, 988 cm^{-1}. (**Z-isomer)** ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (dm, 1 H, *J* =161.2 Hz, H-2), 6.11 (dm, 1 H, *J* = 152.8 Hz, H-3), 3.68 (s, 3 H, $CH_{3\text{-}NMe}$), 3.53 (t, 2 H, *J* = 6.8 Hz, H-14), 3.21 (s, 3 H, $CH_{3\text{-}NMe}$), 2.62 (dm, 2 H, *J* = 123.6 Hz, H-4), 1.76 (p, 2 H, *J* = 7.6 Hz, H-13), 1.67-1.20 (m, 16 H, H-5 to H-12). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7 (d, *J* = 67.7 Hz, C-1), 147.9 (dd, *J* = 67.5, 34.4 Hz, C-3), 118.0 (t, *J* = 67.2 Hz, C-2), 61.5 ($CH_{3\text{-}OMe}$), 45.8 (C-14), 32.7 (C-13), 32.2 ($CH_{3\text{-}NMe}$), 29.7-28.7 (m, 8x CH_2 C-4 to C-13), 27.0 (C-12). IR (neat); 2922, 2853, 1614, 1462, 1331, 1177, 1086, 997 cm^{-1}. HRMS calculated for $[C_{11}{}^{13}C_5H_{30}NO_2Cl + H]^+$: 309.1965, found 309.1966.

[1,2,3,4,5-$^{13}C_5$]-14-Chloro-*N*-methoxy-*N*-methyl-tetradecanamide (30). [1,2,3,4,5-$^{13}C_5$]-(E/Z)-12-Chloro-*N*-methoxy-*N*-methyl-dodec-2-enamide **29** (5.18 g, 16.8 mmol) was dissolved in EtOAc (200 mL). The solution was purged with argon under stirring and a catalytic amount of palladium 10% on charcoal was added. The reaction was stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere overnight. The palladium was removal by filtration over a plug of Celite and the rinsed with EtOAc followed by removal of the solvent under reduced pressure giving the desired product, a colorless oil (5.00 g, 16.1 mmol, 95%). R_f = 0.15 (10% EtOAc in petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3 H, $CH_{3\text{-}OMe}$), 3.53 (t, 2 H, *J* = 6.4 Hz, H-14), 3.18 (s, 3 H, $CH_{3\text{-}NMe}$), 2.40 (dm, 2 H, *J* = 128.0 Hz, H-2), 1.81-1.73 (m 2 H, H-13), 1.60 (dm, 2 H, *J* = 130.4 Hz, H-3), 1.55-1.05 (m, H 18, H-4 to H-12). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.9 (d, *J* = 53.5 Hz, C-1), 61.3 ($CH_{3\text{-}OMe}$), 45.3 (C-14), 32.8-31.6 (m, C-2, C-13 and $CH_{3\text{-}NMe}$), 30.0-29.0 (m, 7x CH_2 C-3 to C-12), 27.0 (CH_2 C-3 to C-12), 25.4-24.1 (m, 2x CH_2 C-3 to C-12). IR (neat) 2922, 2852, 1624, 1462, 1369, 1175, 997 cm^{-1}. HRMS calculated for $[C_{11}{}^{13}C_5H_{32}NO_2Cl + H]^+$: 311.2121, found 311.2123.

[2,3,4,5,6-$^{13}C_5$]-15-Chloro-pentadec-1-ene (31). [1,2,3,4,5-$^{13}C_5$]-14-Chloro-*N*-methoxy-*N*-methyl-tetradecanamide **30** (3.76 g, 12.11 mmol) was dissolved in dry THF (50 mL) and was cooled to -78°C under protected atmosphere. DIBAL-H (1.5 M in toluene, 9.7 mL, 14.5 mmol, 1.2 eq) was added and the mixture was stirred for 1 hour at -78°C. The mixture was quenched with 1 M HCl aq (50 mL) at -78°C and was vigoursly stirred to room temperature. The mixture was diluted with ether and was washed 2x with water and 1 x with brine. The water layers were extracted with ether and the combined organic layers was dried (Na_2SO_4) and concentrated *in vacuo* giving crude [1,2,3,4,5 - $^{13}C_5$]-14-Chloro-tetradecanal, which was used without further purification. Methyltriphenylphosphonium bromide (8.65 g, 24.2 mmol, 2 eq) was suspended in dry THF (120 mL) and NaH (60% in mineral oil, 0.74 mg, 18.2 mmol, 1.5 eq) was added. The reaction mixture was refluxed for 3 h to form the ylide. (mixture turned to yellow) Then the crude [1,2,3,4,5-$^{13}C_5$]-14-Chloro-tetradecanal dissolved in THF (12 mL) was then added to the ylide at 0 °C. The reaction mixture was stirred at room temperature over night. The reaction was diluted with Et_2O and then washed 2x with water and 1x with brine. The water layers were extracted with Et_2O and the organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by column chromatography (hexane) giving a colorless oil (2.48 g, 9.9 mmol, 82%). R_f = 0.95 (pentane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (dm, 1 H, *J* = 150.4 Hz),

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

[5,6,7,8,9-$^{13}C_5$]-[E]-18-Chloro-*N*-(*tert*-butyloxycarbonyl)-D-erytro-sphingosine (32). (2*S*,3*R*)-2-amino-*N*-(*tert*-butyloxycarbonyl)-1,3-dihydroxy-4-pentene (1.5 g, 6.9 mmol, 3 eq) and [2,3,4,5,6-$^{13}C_5$]-15-chloro-pentadec-1-ene **31** (0.57 g, 2.3 mmol) were dissolved in dry DCM (5 mL) and AcOH (26 µL, 0.46 mmol, 0.2 eq). The mixture was flushed with argon before addition of 2nd generation Grubbs catalyst (58 mg, 0.069 mmol, 0.03 eq). The reaction was refluxed for 2 days. The reaction mixture was concentrated *in vacuo* and purified by column chromatography (20% EtOAc in petroleum ether) giving pale brown viscuous oil (882 mg, 2.01 mmol, 86%). R_f = 0.44 (1:1 EtOAc: petroleum ether). Mp 59-60**°**C. $[\alpha]_D^{20}$: + 8.6 (C = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (dm, *J* = 148.0 Hz, H-5), 5.55 (m, 1 H, H-4), 4.28 (m, 1 H, H-3), 3.80 (ddd, 2 H, *J* = 92.8, 11.6, 3.6 Hz, H-1), 3.59 (bs, 1 H, H-2), 3.53 (t, 2 H, *J* = 6.8 Hz, H-18), 3.30-3.00 (m, 2 H, 2x OH), 2.04 (dm, 2 H, *J* = 125.2 Hz, H-6), 1.80-1.72 (m, 2 H, H-17), 1.58-1.05 (m, 27 H, 3x $CH_{3\text{-}tBu}$ and H-7 to H-16). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2 (C=O Boc), 134.1 (d, *J* = 43.4, C-5), 125.1 (d, *J* = 71.7 Hz, H-4), 74.8 (C-3), 62.8 (C-1), 55.6 (C-2), 45.2 (C-18), 32.9-31.7 (m, C-6 and C-17), 29.9-28.4 (m, 10x CH_2 C-7 to C-16 and 3x $CH_{3\text{-}tBu}$), 27.0 (CH_2 C-7 to C-16), 14.1 ($C_{q\text{-}tBu}$). IR (neat) 3390, 2920, 2851, 1688, 1506, 1365 1169, 1053, cm^{-1}. HRMS calculated for $[C_{18}{}^{13}C_5H_{44}NO_4Cl + H]^+$: 439.2958, found 439.2970.

[5,6,7,8,9-$^{13}C_5$] -18-Azido-*N*-(tert-butyloxycarbonyl)-sphinganine (33). Chloro-D-erytro-sphingosine **32** (0.8 g, 1.82 mmol, 1.0 eq) was dissolved in EtOAc (10 mL). The solution was purged with argon under stirring and a catalytic amount of (PtO_2) was added. The reaction was stirred under a flow of hydrogen gas for 30 minutes and was then left under a hydrogen atmosphere overnight. The palladium was removed by filtration over a plug of Celite and then rinsed with EtOAc followed by concentration *in vacuo*. The product was purified by column chromatography (30% EtOAc in petroleum ether) giving a white solid (0.44 g, 1.0 mmol, 86%). R_f = 0.44 (1:1 EtOAc: petroleum ether). Mp 74-75**°**C. $[\alpha]_D^{20}$: + 7.4 (C = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38 (m, 1 H, NH), 3.84 (ddd, 2 H, *J* =97.2, 11.2, 2.8 Hz, H-1), 3.85-3.60 (m, 1 H, H-3), 3.50-3.46 (t, 3 H, *J* = 6.8 Hz, 1 H of H-2 and 2 H of H-18), 2.50 (bs, 1 H, OH), 2.40 (bs, 1 H, OH),1.74-1.68 (m, 2 H, H-17), 1.60-1.05 (m, 35 H, 3x $CH_{3\text{-}tBu}$ and 13x CH_2 H-4 to H-16). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2 (Boc), 74.6 (C-3), 62.8 (C-1), 54.9 (C-2), 45.3 (C-18), 34.6 (C-4), 32.8 (C-17), 29.9-25.7 (12x CH_2 C-5 to C-16 and 3x $CH_{3\text{-}tBu}$), 14.2 ($C_{q\text{-}tBu}$). IR (neat); 3341, 2913, 2847, 1684, 1530, 1171 cm^{-1}. HRMS calculated for $[C_{18}{}^{13}C_5H_{46}NO_4Cl + H]^+$: 441.3115, found 441.3119.

Chloro-*N*-(*tert*-butyloxycarbonyl)-sphinganine (0.4 g, 0.9 mmol, 1.0 eq) was dissolved in dry DMF (4 mL). To the solution was added NaN_3 (0.16 g, 2.7 mmol, 3 eq) and a catalytic amount of NaI and the mixture was heated to 55 **°**C overnight. The mixture was diluted with ether and 2x washed with water and 1x with brine. The water layers were extracted with ether and the organic layers were combined, dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by column chromatography (30% EtOAc in petroleum ether) giving a white solid (0.44 mg, 0.9 mmol, 99%). R_f = 0.40 (1:1 EtOAc: petroleum ether). Mp 62-63 **°**C. $[\alpha]_D^{20}$: + 8.8 (C = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38 (m, 1 H, NH), 3.87 (ddd, 2 H, *J* = 94.8, 11.6, 3.6 Hz, H-1), 3.85-3.60 (m, 1 H, H-2), 3.55-3.48 (m, 1 H, H-3), 3.25 (t, 2 H, *J* = 7.2 Hz, H-18), 2.75 (bs, 1 H, OH), 2.65 (m, 1 H, OH), 1.80-1.00 (m, 37 H, 3x $CH_{3\text{-}tBu}$ and 13x CH_2 H-4 to H-17). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2 (C=O), 74.6 (C-3), 62.8 (C-1), 54.9 (C-2), 51.6 (C-18), 34,4 (C-4), 29.9-25.7 (12x CH_2 C-5 to C-16 and 3x $CH_{3\text{-}tBu}$), 14.2 ($C_{q\text{-}tBu}$). IR (neat) 3341, 2914, 2849, 2097, 1684, 1526, 1169, 906, 729 cm^{-1}. HMRS calculated for $[C_{18}{}^{13}C_5H_{46}N_4O_4 + H]^+$: 448.3519, found 448.3514.

[5,6,7,8,9-$^{13}C_5$] - 18-Azido-sphinganine (22b). To azido-*N*-(tert-butyloxycarbonyl)-sphinganine (0.36 g, 0.8 mmol, 1 eq) was added a mixture of TFA:water (3:1, 8 mL) at 0 °C and stirred for 30 minutes. The mixture was diluted with toluene and concentrated *in vacuo*. The product was purified by column chromatography (9:1 $CHCl_3$:MeOH to 70:27:3 $CHCl_3$:MeOH:H_2O) giving a white TFA-solid (197 mg, 0.56 mmol, 71%). R_f = 0.34 ($CHCl_3$:MeOH:H_2O 70:27:3). $[\alpha]_D^{20} - 3.4$ ($C = 1.0, MeOH$). Mp 82-83 °C. ^{1}H NMR (400 MHz, MeOD); 3.83 (dd, 1 H, *J* = 11.5, 4.0 Hz, H-1$_a$), 3.78 (m, 1 H, H-3), 3.70 (dd, 1 H, *J* = 11.5, 8.8 Hz, H-1), 3.27 (t, 2 H, *J* = 6.8 Hz, H-18), 3.19 (dt, 1 H, *J* = 8.4, 4.0 Hz, H-2), 1.58 (p, 2 H, *J* = 7.2 Hz, H-17), 1.53-1.05 (m, 26 H, H-4 to H-5). ^{13}C NMR (101 MHz, MeOD); 70.34 (C-3), 58.97 (C-1), 58.37 (d, *J* = 3.0 Hz, C-2), 52.45 (C-18), 34.16 (d, *J* = 34.3 Hz, C-4), 30.8-30.0, 29.9, 27.8, 27.4-26.7 (13x CH_2 C-5 to C-17). IR (neat); 3340, 2916, 2849, 2099, 1645, 1215, 1161, 1049, 725 cm^{-1}. HRMS calculated for [$C_{13}{}^{13}C_5H_{38}N_4O_2$ + H]$^+$: 348.2994, found 348.2785.

7.2 References and notes.

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