The synthesis of chemical tools for studying sphingolipid metabolism

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Chapter 6

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

6.1 Introduction

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

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

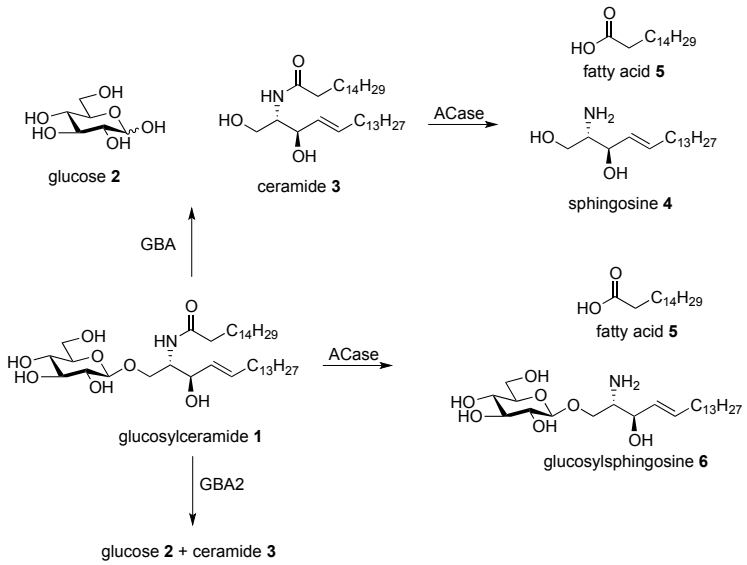


Figure 6.1 Partial overview of metabolism of glucosylceramide **1**. ACase: acid ceramidase; GBA: glucosylceramidase; GBA2: neutral glucosylceramidase.

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

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

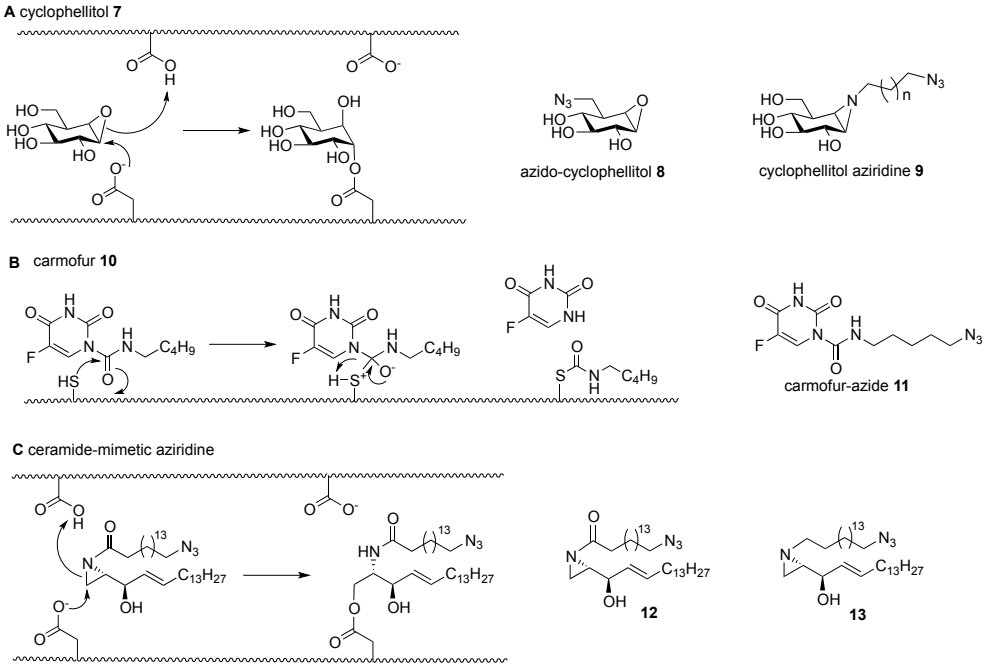


Figure 6.2 A) Mechanism of inhibition of GBA by cyclophellitol **7** and ABP-cyclophellitols **8** and **9**. B) Mechanism of inhibition of acid ceramidase by carmofur **10** and carmofur-azide **11**. C) Possible mechanism of inhibition of GBA/GBA2 and synthetic ceramide aziridine targets **12** and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

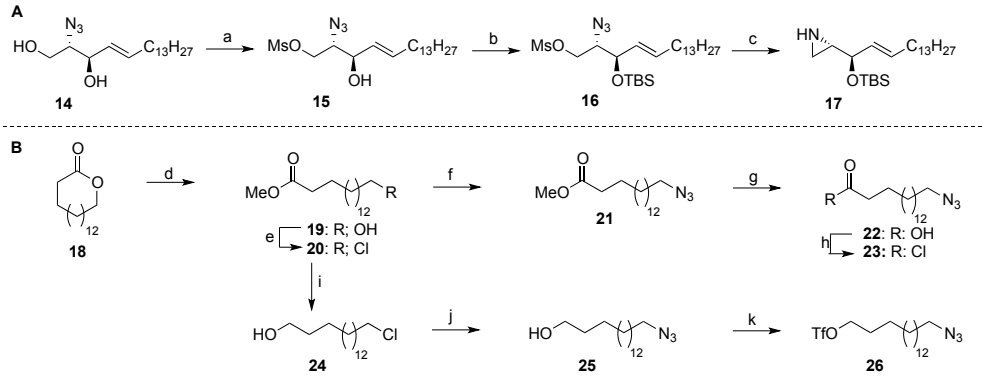
The mode of action of the cyclophellitol-based probes **7**[8,9] and **8** and their derivatives is based on several features. Cyclophellitols are configurational analogues of β-glucopyranose that adopt a $^4H_3$ conformation within the enzyme active site.[12] Once bound, a good leaving group (epoxide-oxygen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

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

6.2 Results and discussion

The construction of aziridine-ceramides **12** and **13** started from the easily accessible azidosphingosine **14**.[13] In the first step, selective mesylation of the primary hydroxyl group in **15** was accomplished by reacting methanesulfonyl chloride and 2,4,6-collidine to *in situ* form a mesylcollidinium species,[14] which due to its steric bulk reacts exclusively with the primarily hydroxyl, yielding compound **15**. The allylic hydroxyl in **15** was masked as the TBS-ether (TBSOTf, 2,4,6-collidine).[15,16] Treatment of the resulting **16** with PPh_3 and water allowed Staudinger reduction of the azide.[17] The *in situ* formed free amine displaced the primary mesyl group in an intramolecular S_N2 nucleophilic substitution to give partially protected aziridine **17** ready for either N-alkylation or N-acylation towards the two target compounds **12** and **13**.

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

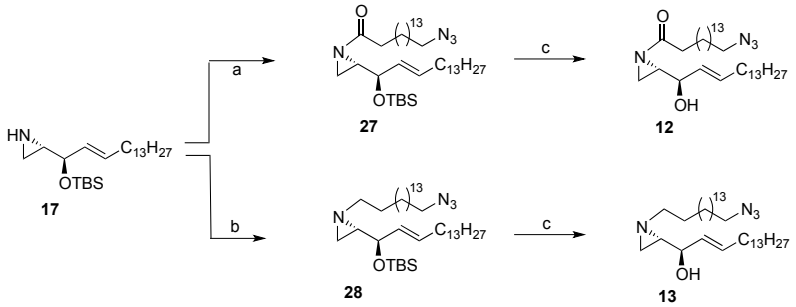


Reagents and conditions: (a) MsCl, 2,4,6-collidine, DCM, 0 °C to 4 °C, 20 h; (b) TBSOTf, 2,4,6-collidine, DCM, 0 °C to 4 °C, 20 h, 65% (over two steps); (c) PPh_3, DIPEA, THF/H_2O (10:1), r.t., 4 h, 59%; (d) NaOMe, MeOH, 60 °C, 3 h, 91%; (e) NCS, PPh_3, THF, r.t., 20 h, 61%; (f) NaN_3, NaI, DMF, 55 °C, 20 h, 94%; (g) LiOH.H_2O, THF/MeOH/H_2O (2:2:1), r.t., 20 h, 61%; (h) oxalyl chloride, DMF, DCM, 0 °C to r.t., 2 h, quant.; (i) $LiAlH_4$ (1 M in THF), THF, 0 °C, 2 h, 86%: (j) NaN_3, NaI, DMF, 55 °C, 20 h, 93%; (k) Tf_2O, pyridine, DCM, 0 °C, 1 h, quant.

The required 16-azido palmitoyl chloride **23** and 16-azido hexadecanoyl triflate **26** for the construction of these target compounds from aziridine **17** were prepared as follows (Scheme 6.1.B). Trans-esterification of cyclohexadecanolide **18** (NaOMe, methanol) provided methyl ester **19** in 91% yield.[18] The primarily hydroxyl group in **19** was transformed into chloride **20** using Appel conditions (*N*-chlorosuccinimide, triphenylphosphine), after which the chloride was displaced by azide (NaN_3, catalytic NaI)

yielding azide **21** in 55% yield over the three steps based on **18**. Next, the methyl ester in **21** was saponified (LiOH in wet THF/MeOH), giving 16-azido-palmitoic acid **22**.[19] Reaction of **22** with oxalyl chloride gave 16-azido-palmitoyl chloride **23**, which was used for aziridine-*N*-acylation without further purification. In order to enable *N*-alkylation with an azide-functionalized C16-alkane, the methyl ester in **20** was treated with excess $LiAlH_4$ to yield alcohol **24**. Next, the chloride in **24** was substituted for an azide (NaN_3, catalytic NaI), giving 16-azido hexadecan-1-al **25** in 94% yield. The primary hydroxyl in **25** was reacted with triflic anhydride and pyridine yielding triflate **26**, which was directly used for *N*-alkylation of aziridine **17**.

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



Reagents and conditions: (a) **23**, Et_3N, DCM, -10 °C to r.t., 3 h, 61%; (b) **26**, DIPEA, DCM, -20 °C, 3 h; ii) MeOH, 62%; (c) TBAF, THF, r.t., 1 h, 62% **12,** 55% **13**.

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

6.3 Conclusion

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

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

6.4 Experimental section

General Remarks. Commercially available reagents and solvents were used as received. DCM and THF were dried and distilled by standard procedures. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out with Silica gel 60 (40–63 µm mesh). IR spectra are reported in cm^{-1}. Optical rotations were measured with an automatic polarimeter (sodium D-line, λ = 589 nm). The enantiomeric purity was determined by HPLC analysis using an OD column (hexane/isopropyl alcohol (98:2), 1 mL/min, UV 254 nm). NMR spectra were recorded on a 400 MHz or 850 MHz spectrometer. Chemical shifts are reported as δ values (ppm), and were referenced to tetramethylsilane (δ = 0.00 ppm) directly in $CDCl_3$, or using the residual solvent peak (D2O). High resolution mass spectra were recorded on a LTQ-Orbitrap (Thermo Finnigan) mass spectrometer equipped with an electrospray ion source in positive mode

(2S,3R,E)-2-azido-3-((tert-butyldimethylsilyl)oxy)octadec-4-en-1-yl methanesulfonate (16). Azidosphingosine **14** (1.11 g, 3.39 mmol, 1 eq.) was dissolved in DCM (38 mL) under an atmosphere of argon and 2,4,6-collidine (4.5 mL, 33.9 mmol, 10 eq.) was added. The mixture was left to stir for 15 minutes at 0 °C. MsCl (0.29 mL, 3.73 mmol, 1.1 eq.) was added and the reaction was left to stir for 21 h at 4 °C, after which the reaction was quenched with water. The mixture was diluted with DCM and washed with 1 M HCl (aq.), sat. aq. $NaHCO_3$ and water. The water layers were extracted with DCM and combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude mesylated product **15** was used in the next step without any further purification. The mesylated sphingosine was then dissolved in DCM (35 mL) and 2,4,6-collidine (4.15 mL, 33.9 mmol, 10 eq.) was added. The mixture was left to stir for 15 minutes at 0 °C. TBSOTf (1.46 mL, 6.8 mmol, 2 eq.) was added and the mixture was left to stir over night at 4 °C. The mixture was diluted with DCM and washed with 1 M HCl (aq.), sat. aq. $NaHCO_3$ and water. The water layers were extracted with DCM and the combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified by column chromatography (5% acetone in pentane) giving a colorless oil (1.12 g, 2.17 mmol, 65%). R_f = 0.35 (5% acetone in pentane); $[\alpha]^{20}_D$: -32.8 (C= 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71(m, 1 H, H-5), 5.41 (dd, 1 H, *J* = 15.8, 7.6 Hz, H-4), 4.31 (dd, 1 H, *J* = 10.8, 3.6 Hz, H-3), 4.19 (dd, 1 H, *J* = 8.0, 5.3 Hz, H-1), 4.10 (dd, 1 H, *J* = 11.0, 8.0 Hz, H-1$_b$), 3.63 (m, 1 H, H-2), 3.04 (s, 3 H, Me_{Ms}), 2.04 (q, 2 H, *J* = 6.8 Hz, H-6), 1.36-1.22 (m, 22 H, H-7 to H-17), 0.89-0.81 (m, 12 H, H-18 and Si_{tBu}) 0.07 (s, 3 H, Si_{Me}) 0.03 (s, 3 H, Si_{Me}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.8 (C-5), 128.2 (C-4), 74.2 (C-3), 68.3 (C-1), 65.3 (C-2), 37.7 (CH_3, Me_{Ms}), 32.36, 32.06, 29.83, 29.80, 29.79 x2, 29.59, 29.50, 29.32, 29.02 (11x CH_2, C-6 to C-17), 25.9 (Si_{tBu}), 22.8 (CH_2, C-6 to C-17), 14.22 (C-18),-2.85 (Si_{Me}), -4.0 (Si_{Me}); IR (neat): 2924, 2855, 2102, 1360, 1252, 1179, 964, 835, 777 cm^{-1}; HRMS calculated for $[C_{25}H_{51}N_3O_4SSi + H]^+$: 518.3450, found 518.3464.

(S)-2-((R,E)-1-((tert-butyldimethylsilyl)oxy)hexadec-2-en-1-yl)aziridine (17). Compound **16** (279 mg, 0.54 mmol, 1 eq.) was dissolved in a mixture of THF/H_2O (10:1, 4 ml). Triphenylphosphine (230 mg, 0.88 mmol, 1.63 eq.) and DIPEA (190 µL, 1.09 mmol, 2 eq.) were added at room temperature and the reaction mixture was stirred for 4 hours. The mixture was diluted with EtOAc and washed with brine. The water layer was extracted with EtOAc and the combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified by column chromatography (silica gel, 4-8% acetone/pentane) giving a colorless oil (116 mg, 0.3 mmol, 59%). R_f = 0.5 (10% acetone/pentane); $[\alpha]^{20}_D$: -30.6 (C= 0.66, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.65 (m, 1 H, H-5), 5.42 (dd, 1 H, *J* =

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

methyl 16-hydroxyhexadecanoate (19). Cyclohexadecanolide **18** (2.6 g, 10.2 mmol, 1 eq.) was dissolved in dry MeOH (60 mL) under an atmosphere of argon. To the solution was added NaOMe (30% in methanol, 9.3 mL, 50 mmol, 5 eq.) was added. The reaction mixture was refluxed for 3 hours at 65 °C after which it was cooled to room temperature. The reaction was quenched with 1 M HCl (aq.) to a pH of 11. The mixture was diluted with EtOAc, washed with sat. aq. $NaHCO_3$. The water layer was extracted with EtOAc and combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified by column chromatography (Silica gel, 1:2 EtOAc/pentane) giving a white solid (2.62 g, 9.11 mmol, 91 %). R_f = 0.75 (1:2 EtOAc/pentane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 3 H, OMe), 3.64 (t, 2 H, *J*= 7.2 Hz, H-16), 2.30 (t, 2 H, *J*= 7.8 Hz, H-2) 1.65-1.51 (m, 4 H, H-3 and H-15), 1.28-1.25 (m, 22 H, H-4 to H-14). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5 (C-1), 63.2 (C-16), 51.6 (C-OMe), 34.3 (C-2), 33.0 (C-15), 29.77 x2, 29.76, 29.74, 29.72, 29.58, 29.39, 29.29, 25.88, 25.10 (12x CH_2, C-3 to C-14); IR (neat): 2918, 2849, 1738, 1161 cm^{-1}.

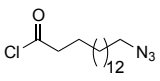
methyl 16-chlorohexadecanoate (20). Compound **19** (2.62 g, 9.1 mmol, 1 eq.) was dissolved in dry THF (60 mL) under an atmosphere of argon. To the solution was added PPh_3 (2.64 g, 10.02 mmol, 1.1 eq.) and NCS (1.39 g, 10.02 mmol, 1.1 eq.). The reaction stirred over night at room temperature. The mixture was diluted with EtOAc and washed with water and brine. The water layers were extracted with EtOAc and combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified by column chromatography in (Silica gel, 0% to 4% EtOAc/pentane) giving a colorless oil (1.69 g, 5.53 mmol, 61%). R_f = 0.8 (2 % EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 3 H, -OMe), 3.53 (t, 2 H, *J*= 7.0 Hz, H-16), 2.30 (t, 2 H, *J* = 7.6 Hz, H-2) 1.76 (p, 2 H, *J* = 7.2 Hz, H-15), 1.62 (m, 2 H, H-3), 1.42 (m, 2 H, H-14), 1.28-1.26 (m, 18 H, H-4 to H-13); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4 (C-1), 51.5 (-OMe), 45.2 (C-16), 34.2 (C-2), 32.8, 29.7 x3, 29.68, 29.65, 29.57, 29.55, 29.36, 29.26, 29.00, 27.0, 25.1 (13x CH_2, C-3 to C15); IR (Neat): 2922, 2853, 1740, 1435, 1169, 721 cm^{-1}.

methyl 16-azidohexadecanoate (21). Chloride **20** (856 mg, 2.8 mmol, 1 eq.) was dissolved in DMF (13 mL) and NaN_3 (580 mg, 8.9 mmol, 3 eq.) and a catalytic amount of NaI were added. The reaction was stirred over night at 55 °C. The mixture was diluted with ether, washed with water and brine and extracted. The water layers were extracted with Ether and the combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, 1-2% acetone in pentane) giving a white solid (791 mg, 2.54 mmol, 94%). R_f = 0.8 (2% acetone/pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.65 (s, 3 H, OMe), 3.24 (t, 2 H, *J*= 7.0 Hz, H-16), 2.29 (t, 2 H, *J* = 7.4 Hz, H-2), 1.63-1.55 (m, 4 H, H-3 and H-15), 1.38-1.26 (m, 22H, H-4 to H-14); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.9 (C-1), 51.3 (C-16), 51.2 (CH_3, C-OMe), 33.9 (C-2), 29.56 x3, 29.52, 29.48, 29.43, 29.39, 29.02, 29.09, 29.08, 29.87, 26.66, 24.87 (13x CH_2, C-3 to C-15); IR (neat): 2922, 2853, 2093, 1740, 1252, 1169 cm^{-1}. HRMS calculated for $[C_{16}H_{31}N_3O_2 + H]^+$: 298.2496, found 298.2509.

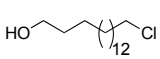
16-azidohexadecanoic acid (22). Compound **21** (791 mg, 2.54 mmol, 1 eq.) was dissolved in a mixture of THF/MeOH/H_2O (2:2:1, 25 mL) and LiOH.H_2O (337 mg, 7.88 mmol, 3.1 eq.) was added. The reaction stirred over night at room temperature, after which it was acidified with 1 M HCl (aq) to a pH of 1-2. The mixture was diluted with EtOAc and extracted with water and brine. The water layers were extracted with EtOAc and combined organic layers were dried ($MgSO_4$), filtered and

concentrated *in vacuo* giving a white solid (446 mg, 1.5 mmol, 59%), which was used without any further purification. R_f = 0.2 (1:1:8 ether/DCM/pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.26 (t, 2 H, *J*= 7.0 Hz, H-16), 2.35 (t, 2 H, *J*= 7.4 Hz, H-2), 1.67-1.56 (m, 4 H, H-3 and H-15), 1.36-1.22 (m, 22 H, H-4 to H-14). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2 (C-1), 51.6 (C-16), 34.0 (C-2), 29.7, 29.6, 29.5, 29.3, 29.2, 29.0, 26.7, 24.9 (13x CH_2, C-3 to C-15); IR (neat): 2913, 2847, 2112, 1699, 1290 cm^{-1}. HRMS calculated for $[C_{15}H_{29}N_3O_2 + H]^+$: 284.2340, found 284.2335.

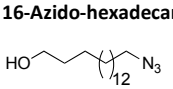
16-azidohexadecanoyl chloride (23). Compound **22** (151 mg, 0.5 mmol, 1 eq.) was dissolved in dry DCM (1.6 mL) and the mixture was cooled to 0 °C. Oxalyl chloride (85 µL, 1 mmol, 2 eq.) was added, followed by 1 drop of DMF, which released gas. The reaction was allowed to warm to room temperature. After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



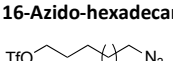
16-Chloro-hexadecan-1-al (24). Methyl ester **20** (0.6 g, 2 mmol, 1 eq) was dissolved in dry diethyl ether (10 mL) under protected atmosphere and cooled to 0 ^{0}C. $LiAlH_4$ (1 M in THF, 2.5 mL, 2.5 mmol, 1.25 eq) was added drop wise and the reaction mixture was stirred for 2 hours at 0 ^{0}C. The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



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



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



1-((S)-2-((R,E)-1-((tert-butyldimethylsilyl)oxy)hexadec-2-en-1-yl)aziridin-1-yl)hexadecan-1-one (27). Aziridine sphingosine **17** (101 mg, 0.26 mmol, 1 eq.) was dissolved in DCM (5 mL) and triethylamine (62 µL, 0.44 mmol, 1.73 eq.) was added. The mixture was cooled to -10 °C. 16-azido-palmitoyl chloride **23** (0.5 mmol, 1.9 eq.) was added drop-wise and the mixture stirred and was allowed to warm to room temperature over 3 h. The solvent was removed *in vacuo* to give a crude product. The crude was purified by column chromatography giving a colorless oil. (silica gel, 0% to 2% acetone/pentane). Yield (155 mg, 0.23 mmol, 90%), R_f = 0.65 (5% acetone/pentane); $[\alpha]^{20}_D$: -27.0 (C= 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81-5.78 (m, 1 H, H-5), 5.58 (dd, 1 H, *J* = 15.6, 8.0 Hz, H-4), 4.20-4.18 (m, 2 H, H-3), 3.37 (t, 2 H, *J* = 7.0 Hz, H-16*) 2.58-2.54 (m, 1 H, H-2), 2.45-2.41 (m, 1 H, H-1_a), 2.20 (d, 1 H, *J* = 3.2 Hz, H-1_b), 2.17-2.13 (m, 2 H, H-6), 1.76-1.69 (m, 4 H, H-2* and H-15*), 1.48-1.37 (m, 46 H, H-7 to H-17 and H-3* to H-14*), 1.00-0.98 (m, 12 H, H-18 and Si_{tBu}), 0.16 (s, 3 H, Si_{Me}), 0.15 (s, 3 H, Si_{Me}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.5 (C-5), 129.5 (C-4), 73.2 (C-3), 51.6 (C-16*), 41.1 (C-2), 36.8 (C-6), 32.3, 32.9, 29.7, 29.6, 29.4, 29.3, 29.2, 28.9, 27.8 (C-1, C-7 to C-17 and C-2* to C-15*), 26.8 (3x CH_3, Si_{tBu}), 25.9, 25.3, 22.8 (C-1, C-7 to C-17 and C-2* to C-15*), 14.2 (C-18), -4.0 (2x CH_3, Si_{Me}); IR (neat): 2922, 2853, 2093, 1703, 1464, 1250, 970, 835 cm^{-1}. HRMS calculated for $[C_{39}H_{74}N_4O_2Si + H]^+$: 675.5974, found 675.5998.

1-((S)-2-((R,E)-1-hydroxyhexadec-2-en-1-yl)aziridin-1-yl)hexadecan-1-one (12). Aziridine-ceramide **27** (0.23 mmol, 1 eq.) was dissolved in THF (1.5 mL) and TBAF (1M in THF, 275 µL, 0.28 mmol, 1.2 eq.) was added. The reaction stirred for 1 h. at room temperature. The mixture was diluted with EtOAc/H_2O and washed with brine and extracted. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The yielded product was purified by column chromatography giving a colorless oil. (Silica gel, 10% to 20% acetone/pentane) Yield (81 mg, 0.14 mmol, 61%), R_f = 0.5 (10% acetone/pentane), $[\alpha]^{20}_D$: -16.2 (C= 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84-5.75 (m, 1 H, H-5), 5.44 (dd, 1 H, *J* = 15.6, 8.0 Hz, H-4), 5.08 (t, 1 H, *J* = 10.4 Hz, H-3), 3.26 (t, 2 H, *J* = 9.2 Hz, H-34), 2.32 (t, 2 H, *J* = 10 Hz, H-20), 2.24-2.18 (m, 1 H, H-2), 2.05-2.03 (m, 2 H, H-6), 1.78 (d, 1 H, *J* = 7.6 Hz, H-1), 1.62-1.55 (m, 5 H, H-1, H-21 and H-33), 1.26 (s, 44 H, H-7 to H-17 and H-22 to H-32), 0.88 (t, 3 H, *J* = 8.8 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1 (C-19), 136.6 (C-5), 124.9 (C-4), 75.6 (C-3), 51.7 (C-34), 34.7 (C-20), 32.5, 32.2 (25C, C-1, C-21 to C-33), 32.1 (C-2), 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.0, 26.9, 25.2, 23.1, 22.9 (25C, C-1, C-21 to C-33), 14.3 (C-18). IR (Neat): 2914, 2849, 2095, 1726, 1468, 1177, 968 cm^{-1}; HRMS calculated for $[C_{34}H_{64}N_4O_2 + H]^+$: 561.5109, found 561.5138.

Alkylaziridine sphingosine (28). Aziridine **17** (58 mg, 0.15 mmol, 1.0 eq) was dissolved in dry DCM (1.5 mL) under protected atmosphere and cooled -20 °C. DIPEA (29 µL, 0.16 mmol, 1.1 eq) was added followed by addition of triflate **26** (1 M in DCM, 1.6 mL, 0.16 mmol, 1.1 eq) and was left stirring for 3 hours. The reaction mixture was quenched with MeOH (0.1 mL) and washed with water and brine. The water layers were extracted with DCM and combined organic layers were dried ($MgSO_4$) filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (pentane to 2% acetone in pentane) giving a colorless oil (63 mg, 0.09 mmol 62%). $[\alpha]^{20}_D$: 44 (C= 0.5, $CHCl_3$); R_f = 0.72 (5% acetone in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (m, 1 H, H-5), 5.53 (dd, 1 H, *J* =12.4 6.0 Hz, H-4), 3.55 (t, 1 H, *J* = 6.8 Hz, H-3), 3.25 (t, 2 H, *J* = 7.2 Hz, H-16*), 2.43 (m, 1 H, H-1_a*), 2.01 (q, 1 H, *J* = 7.2 Hz, H-6), 1.93 (m, 1 H, H-1_b*), 1.66 (d, 1 H, *J* = 3.2 Hz, H-1_a), 1.61 (m, 1 H, H-2*), 1.41-1.25 (m, 48 H, H-1_b, H-2, H-7 to H-17 and H-3* to H-15*), 0.88 (m, 12 H, H-18 and Si_{tBu}), 0.02 (s, 3 H, Si_{Me}), 0.01 (s, 3 H, Si_{Me}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.96 (C-5), 127.79 (C-4), 76.13 (C-3), 61.39 (C-1*), 51.62 (C-16*), 44.80 (C-2), 33.48, 32.33, 32.08, 29.97, 29.70, 29.64, 29.41, 29.36, 29.31, 28.98, 27.57, 26.86, 26.71, 26.86, 26.71 (C-1, C-6 to C-17 and C-2* to C-15*), 22.84 (Si_{tBu}), 18.38 ($C_{q\text{-}Si\text{-}tBu}$), 14.27 (C-18), -4.29, -4.39 (2x $C_{Si\text{-}Me}$); IR (neat); 2922, 2852, 2094, 1463, 1249, 1066, 835, 775 cm^{-1}; HMRS calcd for $[C_{40}H_{80}N_4OSi + H]^+$: 661.6181, found 661.6206.

Alkylaziridine sphingosine (13). The alkylated aziridine **28** (52 mg, 0.08 mmol, 1.0 eq) was dissolved in dry THF (0.8 mL) under an atmosphere of Argon. TBAF (1 M in THF, 0.1 mL, 0.1 mmol, 1.25 eq) was added and the reaction was stirred for 2 hours at room temperature. The reaction was diluted with EtOAc and washed with water and Brine. The water layers were extracted with EtOAc and combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified by silica column chromatography (5 % Acetone in Pentane) giving waxy white solid (24 mg, 0.044 mmol, 55%). $[\alpha]^{20}_D$: 25 (C= 0.25, $CHCl_3$); R_f = 0.56 (10% Acetone in Pentane); ^{1}H NMR (400 MHz, $CDCl_3$): 5.73 (dt, 1 H, *J* = 15.6, 8.0 Hz, H-5), 5.38 (dd, 1 H, *J* = 15.6, 7.6 Hz, H-4), 4.15 (m, 1 H, H-3), 3.25 (t, 2 H, *J* = 6.8 Hz, H-16*), 2.41 (m, 1 H, H-1$_a$*), 2.19 (m, 1 H, H-1$_b$*), 2.03 (m, 2 H, H-6), 1.81 (d, 1 H, *J* = 3.2 Hz, H-1$_a$), 1.62-1.53 (m, 5 H, H-1$_b$, H-2* and H-15*),)1.35-1.25 (m, 45 H, H-2, H-7 to H-17 and H-3* to H-14*), 0.88 (t, 3 H, *J* = 7.2 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) : 134.00 (C-5), 129.39 (C-4), 70.05 (C-3), 60.28 (C-1*), 51.63 (C-16*), 42.71 (C-2), 32.47, 32.07, 29.81, 29.77, 29.69, 29.65, 29.54, 29.51, 29.35, 29.30, 29.24, 28.98, 28.77, 27.47, 26.86, 25.66, 22.84 (C-1, C-6 to C-17 and C-2* to C-15*), 14.27 (C-18); IR (neat) 2921, 2850, 2094, 1467, 1177, 968 cm^{-1}. HRMS calculated for $[C_{34}H_{66}N_3O + H]^+$: 547.5317, found 547.5339.

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