The synthesis of chemical tools for studying sphingolipid metabolism

Wisse, P.

Chapter 3

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

3.1 Introduction

In Chapter 2 the synthesis of carbon-13-labeled glucosylsphingosine **3** was described. Key step in this synthesis comprised glycosylation of *N*-Boc-protected sphingosine **1** using imidate **2** as the donor glucoside (Figure 3.1).[1] During the research that led to the identification of 4,6-silylidene donor **2**, perbenzoylated glucopyranosyl imidates **4** and **5** were assessed as well. In these studies it was found that the use of stoichiometric $BF_3.OEt_2$ as the Lewis acid to activate these donors in the presence of *N*-Boc-sphingosine **1** did not

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

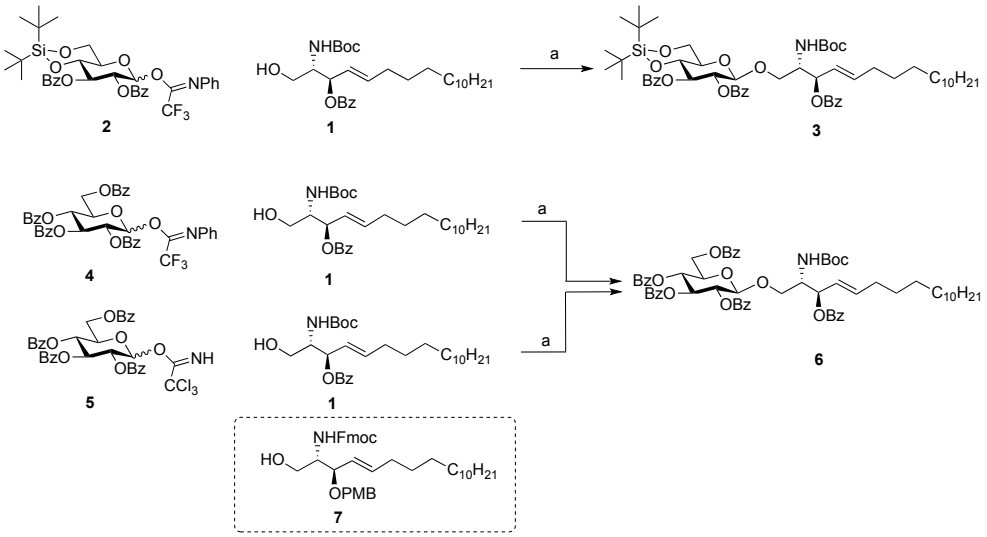


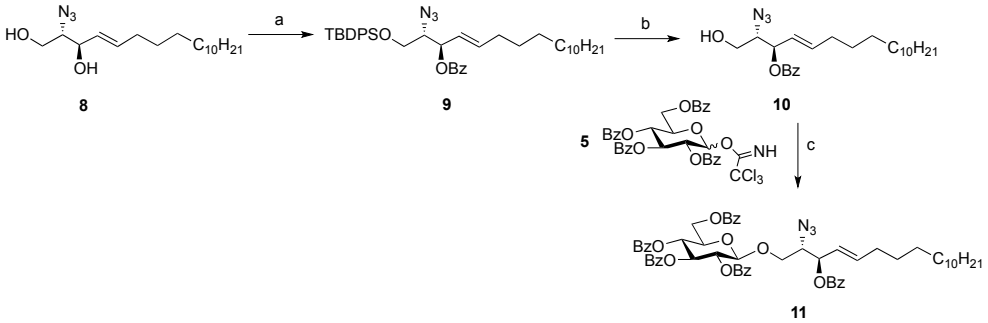
Figure 3.1. *N*-Boc-protected sphingosine **1** could be glucosylated with donor imidate **2**, but not with the comparatively less reactive donor glucosides **4** and **5**. Conditions: a) BF_3OEt_2, DCM, 0 °C, 1 h, **3**: 49%; **6**: no productive yield from either **4** or **5**.

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

3.2 Results and discussion

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

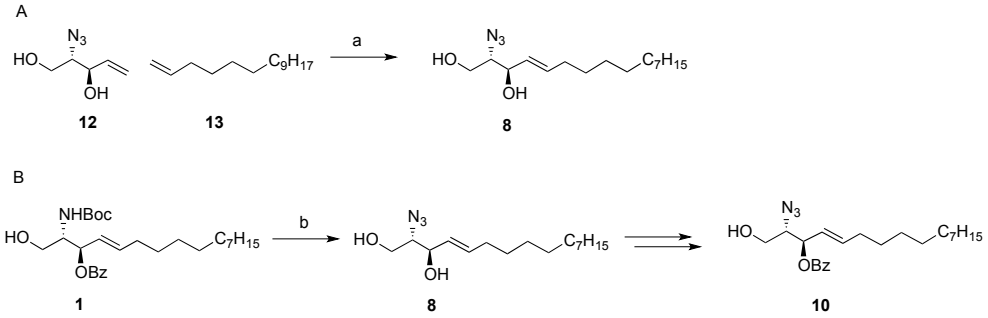
Scheme 3.1. Glycosylation of azide-protected sphingosine.



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

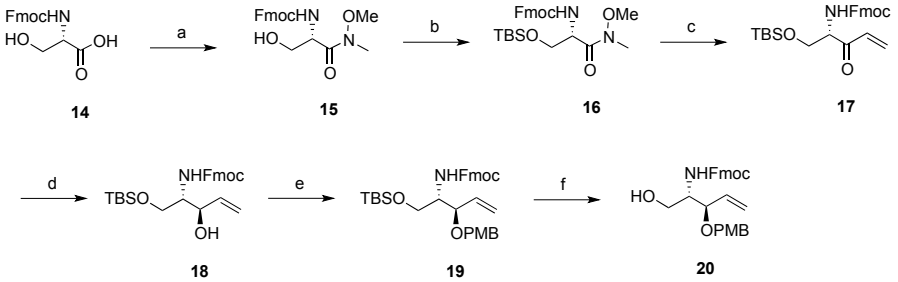
However and as was shown previously, the azide in terminal alkene **12** prevented its effective cross-metathesis with terminal alkene **13** (Scheme 3.2).[5,6] Cross-metathesis of carbon-13-enriched alkene **13** to an appropriately protected form of alkene **12**, which itself can be prepared in an enantiomerically pure form from serine, is the strategy of choice for the preparation of neutron-encoded sphingosine bases, as was described in Chapter 2.[1] On paper, one could produce $^{13}C_5$-enriched, N-Boc protected sphingosine **1**, remove both O-benzoyl protection and N-Boc protection (in this order to avoid acyl migration from O to N), perform a diazo transfer, and then install the secondary benzoyl following the scheme as depicted for sphingosine **10**. Performing this, rather lengthy, protective group manipulation scheme on a (comparatively expensive) carbon-13-enriched compound however is suboptimal. Therefore, an alternative, partially protected sphingosine acceptor, that could be prepared through the cross-metathesis scheme, and that at the same time would be able to withstand rather acidic glycosylation conditions, was required. Such a sphingosine derivative was found in compound **7**, in which the amine is masked with an Fmoc group and the secondary alcohol as the *para*-methoxybenzyl ether.[5,6]

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



Reagents and conditions: (a) Grubbs 2nd generation catalyst, AcOH, CH_2Cl_2, reflux, 48 h; (b) NaOMe, MeOH, r.t., 20 h; (ii) KOH, H_2O, r.t., 20 h; (iii) TFA, H_2O, 0 °C, 30 min; (iv) imidazole-1-sulfonyl azide hydrochloride, K_2CO_3, $CuSO_4$. 5 H_2O, MeOH. r.t., 20 h.

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



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

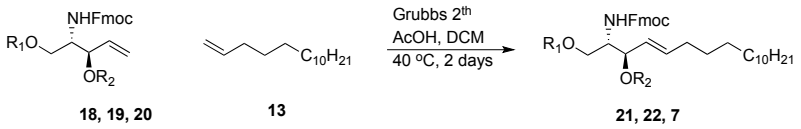
Reagents and conditions: (a) *N,O*-dimethylhydroxylamine hydrochloride, EDC.HCl, DIPEA, DCM, HOBt.H_2O, 0 °C to r.t., 20 h; (b) TBS-Cl, NMM, DMF, 0 °C to r.t., 20 h, 75% over two steps; (c) (i) vinylmagnesium bromide, THF, 0 °C, 1 h, 67%; (d) $LiAlH(OtBu)_3$, EtOH, -78 °C, 5 h, 73%; (e) PMB-*N*-phenyltrifluoroimidate, camphor-10-sufonic acid, toluene, 0 °C to r.t., 20 h, 58%; (f) TBAF, acetic acid, THF, r.t., 20 h, 94%.

In the next step, Weinreb amide **16** was treated with vinylmagnesium bromide to give the α,β-unsaturated ketone **17**. The stable tetrahedral intermediate that is formed upon addition of the Grignard reagent and that collapses only during work-up (addition of 2M HCl) ensures that a second Grignard addition is prevented.[8,9] Ketone **17** was then reduced in a stereoselective fashion to give allylic alcohol **18** using $LiAlH(OtBu)_3$ as reducing agent.[7,10] The alcohol moiety in **18** was next protected as the *para*-methoxybenzyl ether

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

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

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

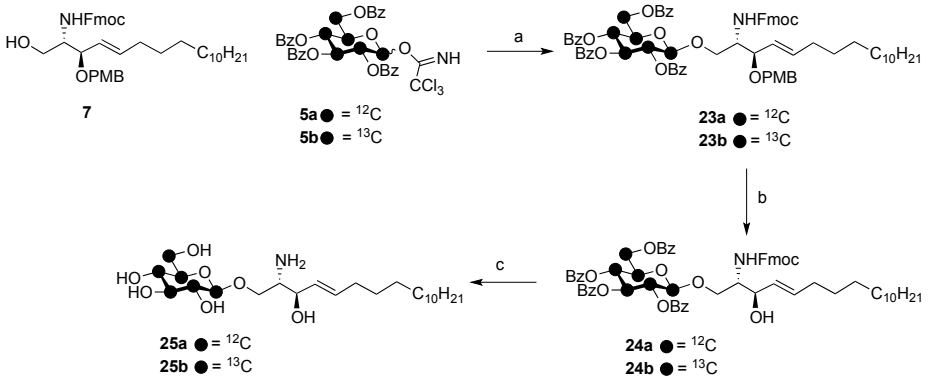


18, 19, 20 **13** **21, 22, 7**

entry	pentene	R_1	R_2	Product	Yield (%)
1	**18**	TBS	H	**21**	62
2	**19**	TBS	PMB	**22**	18
3	**20**	H	PMB	**7**	65

Both because of the (slightly) higher yield and because the partially protected sphingosine produced from **20** would allow modification of the primary alcohol directly, the process summarized in entry 3 is the most suitable for preparation of $^{13}C_5$-enriched sphingosine derivatives. For this to become feasible, the compatibility of Fmoc-sphingosine **7** in glycosylation events needed to be established. In a first attempt, glycosylation of **7** with imidate **5** under the agency of BF_3OEt_2 (Scheme 3.4) proved abortive. Switching to the stronger Lewis acid, TMSOTf (10 mol%) led to the formation of the desired, fully protected glycosylated sphingosine **23** in good yield, thus revealing that switching from *N*-Boc-protection to *N*-Fmoc-protection, as was hypothesized, pays off.[5,13] Removal of the *para*-methoxybenzyl group (10% TFA in DCM), followed by global removal (sodium hydroxide in a mixture of methanol and methylene chloride) of the benzoyl and *N*-Fmoc protective groups yielded β-glucosylsphingosine **25** in 80% yield based on **23**. All spectroscopic and analytical data on **25** were in full agreement with the data obtained on the same compound as synthesized in Chapter 2.[1] In a similar vein, but now starting from $^{13}C_6$-glucose donor **5b**, the corresponding isotopically enriched glucosylsphingosine **25b** could be prepared.

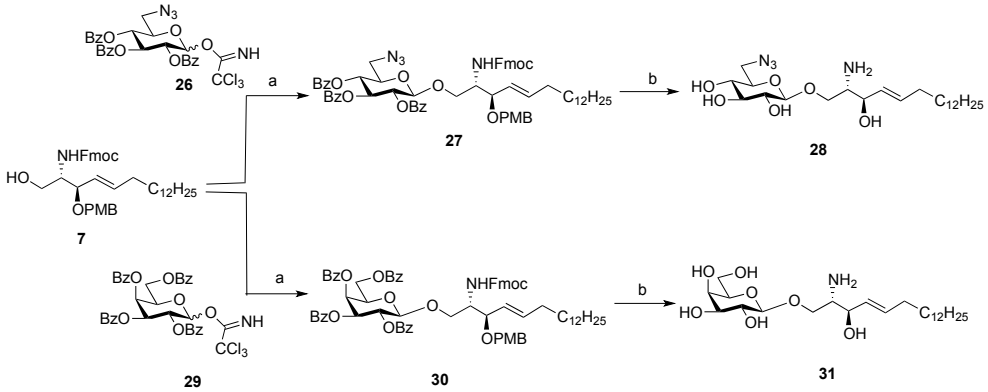
Scheme 3.4 Synthesis of $^{13}C_6$-glucosylsphingosine **25a/b** using *N*-Fmoc-protected sphingosine **7**.



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

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

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



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

3.3 Conclusion

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

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

3.4 Experimental section

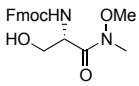
General Remarks: Commercially available reagents and solvents (Acros, Fluka, or Merck) were used as received, unless otherwise stated. CH_2Cl_2 and THF were freshly distilled before use, over P_2O_5 and Na/benzophenone, respectively. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide. Traces of water were removed from starting compounds by co-evaporation with toluene. All moisture-sensitive reactions were carried out under an argon atmosphere. Molecular sieves (3 Å) were flame-dried before use. Column chromatography was carried out using forced flow of the indicated solvent systems on Screening Devices silica gel 60 (40–63 µm mesh). Size-exclusion chromatography was carried out on Sephadex LH20 (MeOH/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 molybdeen/cerium sulphate solution [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)6{\cdot}2H_2O$ (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 re- corded 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 %).

General procedure for the glycosylation of *N*-Fmoc-protected sphingosine 7 and trichloro-imidate donors. Protected sphingosine (1 eq) and imidate-donor (1.5 eq) were co-evaporated twice with toluene before it was dissolved in dry DCM (0.1 M) under protected atmosphere. To the solution was added activated 4 Å molsieves and was stirred for 30 minute. The reaction mixture was cooled to -20 °C, followed by activation with TMSOTf (10% mol, based on sphingosine). The reaction was stirred at -20 °C until, according TLC, all sphingosine acceptor was consumed (approximately 1 hour). When the reaction was complete, the reaction mixture was neutralized with triethylamine and filtered over Celite. The reaction was concentrated *in vacuo* followed by purification by silica gel chromatography or with size exclusion column giving protected glycosylsphingosine.

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

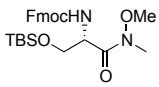
at room temperature. The reaction was according LC-MS finished and the reaction was neutralized with AcOH and concentrated *in vacuo.* The crude product was purified with silica column chromatography (10% MeOH in chloroform to 3:27:70 H_2O:MeOH:$CHCl_3$) giving fully deprotected sphingosine.

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



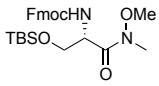
solution of DIPEA (8.2 mL, 46.9 mmol, 0.9 eq) in DCM (300 mL) was added Fmoc-L-Serine **14** (17.1 g, 52.2 mmol, 1 eq). The mixture was stirred under argon at 0 °C. EDC.HCl (12 g, 62.6 mmol, 1.2 eq) and HOBt.H2O (9.58 g, 62.6 mmol, 1.2 eq) were added and stirred for 10 minutes, followed by addition of Weinreb salt (6.12 g, 62.6 mmol, 1.2 eq) and DIEA (4.5 mL, 26.1 mmol, 0.5 eq). The reaction was stirred over night, which was allowed to reach room temperature. The mixture was washed 2x 2M HCl, 2x sat. aq. $NaHCO_3$ and brine. All water layers were extracted with DCM and the combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo* giving resulting oil **10**, which was used in the next step without further purification. R_f = 0.39 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2 H *J* = 7.5 Hz, CH_{Fmoc}), 7.64–7.56 (m, 2 H, CH_{Fmoc}), 7.40 (t, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 7.31 (t, 2 H, CH_{Fmoc}), 5.92 (d, 1 H, *J* = 8.3 Hz, NH), 4.87 (m, 1 H, H-2), 4.40 (d, 2 H, *J* = 7.1 Hz, $CH_{2\text{-}Fmoc}$), 4.22 (t, 1 H, *J* = 7.0 Hz, CH_{Fmoc}), 3.86 (s, 2H, H-1), 3.78 (s, 3 H, $CH_{3\text{-}OMe}$), 3.25 (s, 3 H, $CH_{3\text{-}NMe}$), 2.52 (bs, 1 H, OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.0 (C=O_{Fmoc}) 143.93, 143.82, 2x 141.39 ($C_{q\text{-}Fmoc}$), 141.46 (C-3), 127.84, 127.21, 125.25, 120.10 (4x CH_{Fmoc}), 67.35 ($CH_{2\text{-}Fmoc}$), 63.70 (C-1), 61.79 ($CH_{3\text{-}OMe}$), 52.88 (C-2), 47.26 (CH_{Fmoc}) 31.91 ($CH_{3\text{-}OMe}$); IR (neat): 3412, 3315, 2941, 1714, 1448, 1263, 1053 cm^{-1}; HRMS calculated for $[C_{20}H_{22}N_2O_5 + H]^+$: 371.1537, found 371.1531.

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



Crude product **15** was dissolved in dry DMF (500 mL) under protected atmosphere and cooled to 0 °C. To the solution was added TBDMS-Cl (10.2 g, 67.6 mmol, 1.3 eq) followed by addition of NNM (6.3 mL, 57.2 mmol, 1.1 eq). The mixture was stirred over night, which was allowed to reach room temperature. The reaction mixture was diluted with H_2O was added and was extracted twice with diethyl ether. The organic layers were combined, washed with brine, dried with $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude oil was purified with silica gel chromatography (0-10% EtOAc in pentane), which gave yellow oil **16** (18.9 g, 39.0 mmol, 75% over two steps). R_f = 0.95 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 7.62 (t, 2 H, *J* = 8.2 Hz, CH_{Fmoc}), 7.40 (t, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 7.32 (t, 2 H, *J* = 7.5 Hz, CH_{Fmoc}), 5.72 (d, 1 H, *J* = 8.7 Hz, NH), 4.85 (m, 1 H, H-2), 4.37 (d, 2H, *J* = 7.2 Hz, $CH_{2\text{-}Fmoc}$), 4.25 (t, 1 H, *J* = 7.3 Hz, CH_{Fmoc}), 3.96–3.83 (m, 2 H, H-1), 3.77 (s, 3 H, $CH_{3\text{-}OMe}$), 3.25 (s, 3 H, $CH_{3\text{-}NMe}$), 0.90 (s, 9 H, TBS_{tBu}), 0.06 (s, 6 H, 2x TBS_{Me}); ^{13}C NMR (101 MHz $CDCl_3$) δ 168.0 (C-3), 156.0 (C=O_{Fmoc}) 143.93, 143.82, 2x 141.39 ($C_{q\text{-}Fmoc}$), 127.82, 127.20, 125.34, 120.09, (4x CH_{Fmoc}) 67.27 ($CH_{2\text{-}Fmoc}$), 63.48 (C-1), 61.65 ($CH_{3\text{-}OMe}$), 53.16 (C-2), 47.28 (CH_{Fmoc}), 32.62 ($CH_{3\text{-}NMe}$), 25.93 (TBS_{tBu}), 18.26 ($TBS_{tBu\text{-}q}$), -5.35 (TBS_{Me}); IR (neat) 3305, 3057, 2924, 1712, 1450, 1053 cm^{-1}; HRMS calculated for $[C_{26}H_{36}N_2O_5Si + H]^+$: 485.2401, found 485.2404.

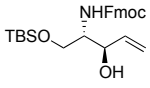
(9H-fluoren-9-yl)methyl (S)-(1-((*tert*-butyldimethylsilyl)oxy)-3-oxopent-4-en-2-yl)carbamate (17). Silylated



protected **16** (4.8 g, 10 mmol, 1 eq) was in dry THF (20 mL) under protected atmosphere and cooled to 0 °C. To the solution was added slowly vinyl MgBr (1 M in THF, 40 mL, 40 mmol, 4 eq) and the reaction was stirred for one hour at 0 °C. The reaction mixture was quenched by slowly adding it to cold 2M HCl (200 mL) and quickly extracted twice with EtOAc. The organic layers were combined, washed with brine, dried with $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was purified with silica gel chromatography (2.5-10% EtOAc in pentane), which gave a white solid **17** (3 g, 6.7 mmol, 67%). R_f = 0.75 (15% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 2 H, *J* = 7.5 Hz, H_{Arom}), 7.62 (t, 2 H, *J* = 6.8 Hz, H_{Arom}), 7.41 (t, 2 H, *J* = 7.5 Hz, H_{Arom}), 7.32 (t, 2 H, *J* = 7.5, H_{Arom}), 6.57 (dd, 1 H, *J* = 17.2, 10.8 Hz, H-4), 6.39 (d, 1 H, H-5_a), 5.90–5.83 (m, 2 H, H-5_b + NH), 4.70 (m, 1 H, H-2), 4.39 (d, 2 H, *J* = 7.2 Hz, $CH_{2\text{-}Fmoc}$), 4.24 (t, 1 H, *J* = 7.2 Hz, CH_{Fmoc}), 4.05 (d, 1 H, *J* = 10.2 H-1_a), 3.90 (dd, 1 H, *J* = 10.4, 4.0 H-1_b), 0.86 (s, 9 H, TBS_{tBu}), 0.02 (s, 3 H, TBS_{Me}), 0.01 (s, 3 H, TBS_{Me}); ^{13}C NMR (101 MHz $CDCl_3$) δ 196.9 (C-3) 155.96 (C=O_{Fmoc}), 144.05, 143.93, 2x 141.44 ($C_{q\text{-}Fmoc}$), 133.14 (C-4), 129.9 (C-5), 127.85, 127.21, 125.30, 120.12 (4x CH_{Fmoc}), 67.26 ($CH_{2\text{-}Fmoc}$), 63.47 (C-1), 60.05 (C-2), 47.31 (CH_{Fmoc}), 25.86 (TBS_{tBu}), 18.33 ($TBS_{tBu\text{-}q}$), -5.44 (TBS_{Me}); IR (neat) 2953, 2927, 2854, 1699, 1500, 1450,

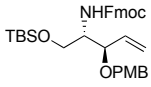
1251, 1026 cm^{-1}; HRMS calculated for $[C_{26}H_{33}N_1O_4Si + H]^+$: 452.2799, found 452.2783.

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



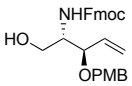
Allic ketone **17** (3.0 g, 6.7 mmol, 1 eq) was dissolved in EtOH (500 mL) under an protected atmosphere and cooled to -78 °C. To the solution was added $LiAlH(OtBu)_3$ (3.7 g, 14.7 mmol, 2.2 eq) and for 5 hours at -78 °C and was quenched with 0.1 M HCl (175 mL) at the same temperature. The mixture was quickly extracted twice with EtOAc and the combined organic layers were washed with 0.1 M HCl and brine, dried with $MgSO_4$, filtered and concentrated *in vacuo*. The crude oil was purified with silica gel chromatography (2.5-10% EtOAc in pentane) giving a white solid **18** (2.2 g, 4.9 mmol, 73%) and starting material (0.7 g, 1.6 mmol, 23 %). R_f = 0.52 (15% EtOAc in pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 2 H, *J* = 7.5 Hz, H_{Fmoc}), 7.58 (m, 2 H, H_{Fmoc}), 7.40 (t, 2 H, *J* = 7.5 Hz, H_{Fmoc}), 7.31(m, 2 H, H_{Fmoc}), 5.94 (m, 1 H, H-4), 5.56 (d, 1 H, *J* = 8.6 Hz, NH), 5.41 (d, 1 H, *J* = 17.1 Hz, H-5_a), 5.27 (d, 1 H, *J* = 10.6 Hz, H-5_b), 4.39 (d, 2 H, *J* = 7.2, Hz, $CH_{2\text{-}Fmoc}$), 4.31 (m, 1 H, H-3), 4.24 (t, 1 H, *J* = 7.0 Hz, CH_{Fmoc}), 3.97 (dd, 1 H, *J* = 10.4, 2.8 Hz, H-1_a), 3.78 (d, 1 H, *J* = 10.2, 3.2 Hz, H-1_b), 3.37 (m, 1 H, H-2), 3.38 (d, 1 H, *J* = 8.4 Hz, OH), 0.91 (s, 9 H, TBS_{tBu}), 0.07 (s, 6 H, 2x TBS_{Me}); ^{13}C NMR (101 MHz $CDCl_3$) δ 156.3 ($C{=}O_{Fmoc}$), 144.06, 143.97, 2x 141.44 (4x $C_{q\text{-}Fmoc}$) 137.34 (C-4), 127.83, 127.18, 125.22, 120.14 (4x CH_{Fmoc}), 116.36 (C-5), 74.88 (C-3), 66.95 ($CH_{2\text{-}Fmoc}$), 62.35 (C-1), 55.19 (C-2), 47.34 (CH_{Fmoc}), 25.94 (TBS_{tBu}), 18.24 ($TBS_{tBu\text{-}q}$), -5.50 (TBS_{Me}); IR (neat): 3439, 2927, 2854, 1705, 1251, 1080 cm^{-1}; HRMS calculated for $[C_{26}H_{35}NO_4Si + H]^+$: 453.2343, found 453.2348.

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



Allylic alcohol **18** was dissolved in dry DCM toluene (1 mL) under protected atmosphere, followed by addition of activated 4 Å molsieves and PMB-fluoro imidate (0.11 g, 0.36 mmol, 1.5 eq) The mixture was stirred for 30 minutes, after which was cooled to 0 °C before addition of 10-Camphorsulphonic acid (7 g, 0.024 mmol, 0.1 eq). The mixture was stirred over night and was allowed reaching room temperature. The mixture was diluted with DCM and filtered over Celite, washed with sat. aq. $NaHCO_3$ (aq) and brine. The water layers were extracted with DCM and the combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified with silica gel chromatography (2.5-10 % EtOAc in pentane) giving a slightly yellow solid **19** (0.08 g, 0.13 mmol, 58%). R_f = 0.5 (10% EtOAc in pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, 2 H, *J* = 7.5 Hz, H_{Fmoc}), 7.57 (m, 2 H, H_{Fmoc}), 7.39 (t, 2 H, *J* = 7.5 Hz, H_{Fmoc}), 7.29 (t, 2 H, *J* = 7.5 Hz, H_{Fmoc}), 7.23 (d, 2 H, *J* = 7.5 Hz, H_{PMB}), 6.86 (d, 2 H, *J* = 8.0 Hz, H_{PMB}), 5.83 (m, 1 H, H-4), 5.52 (d, 1 H, *J* = 8.4, NH), 5.42-5.34 (m, 2 H, H-5_a and NH), 5.06 (d, 1 H, *J* = 9.7 Hz, H5_b), 4.54 (d, 1 H, *J* = 11.3 Hz, $CH_{2\text{-}PMB\text{-}a}$), 4.34-4.12 (m, 5 H, $CH_{2\text{-}Fmoc}$, $CH_{Fmoc,}$ $CH_{2\text{-}PMB\text{-}b}$ and H-3), 3.96 – 3.87 (m, 2 H, H-1_a and H-2), 3.78 (s, 3 H, OMe_{PMB}), 3.65 (dd, 1H, *J* = 10.0, 4.1 Hz, H-1_b), 0.90 (d, 9 H, *J* = 2.9 Hz, TBS_{tBu}), 0.06 (s, 3 H, TBS_{Me}), 0.05 (s, 3 H, TBS_{Me}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.32 ($C{=}O_{Fmoc}$), 144.02, 141.37 (4x $C_{q\text{-}Fmoc}$), 136.02 (C-4), 130.3 (CH_{PMB}), 129.4, 127.74, 127.48, 125.20, 120.12 (4x $C_{q\text{-}Fmoc}$), 116.33 (C-5), 113.8 (CH_{PMB}), 74.81 (C-3), 70.50 ($CH_{2\text{-}PMB}$), 66.98 ($CH_{2\text{-}Fmoc}$), 62.24 (C-1), 55.39 (OMe_{PMB}) 55.13 (C-2), 47.30 (CH_{Fmoc}), 25.92 (TBS_{tBu}), 18.22 ($TBS_{tBu\text{-}q}$), -5.50 (TBS_{Me}); IR (neat): 3444, 3317, 3070, 2950, 2854, , 1242 cm^{-1}; HRMS calculated for $[C_{34}H_{43}NO_5Si + H]^+$: 573.2919, found 573.2923.

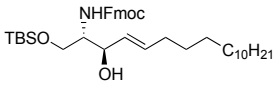
(9H-fluoren-9-yl)methyl ((2S,3R)-1-hydroxy-3-((4-methoxybenzyl)oxy)pent-4-en-2-yl)carbamate (20). To a



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

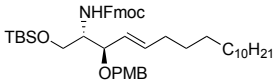
H, *J* = 11.6 Hz, H-1$_b$), 3.76 (s, 3 H, OMe$_{PMB}$), 3.68 (m, 1 H, H-2), 3.63 (dd, 1 H, *J* = 11.2, 2.4 Hz, H-1$_b$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.31 (C=O$_{Fmoc}$), 144.06, 141.41 (4x C$_{q\text{-}Fmoc}$), 136.09 (C-4), 129.92 (CH$_{PMB}$), 127.83, 127.19, 125.23 (C-4), 125.29, 120.08 (4x CH$_{Fmoc}$) 116.37, (C-5), 114.03 (CH$_{PMB}$), 74.89 (C-3), 70.46 (CH$_{2\text{-}PMB}$), 66.96 (CH$_{2\text{-}Fmoc}$), 62.20 (C-1), 55.36 (OMe$_{PMB}$), 55.31 (C-2) 47.30 (CH$_{Fmoc}$); IR (neat): 3487, 3340, 3050, 2880, 1674, 1250 cm^{-1}; HRMS calculated for $[C_{28}H_{29}NO_5 + H]^+$: 460.2054, found 460.2059.

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



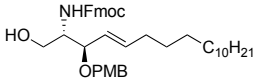
To a solution of **18** (1.1 g, 2.45 mmol, 1 eq) in DCM (15 mL), 1-pentadecene (2.12 mL, 4.9 mmol, 2 eq) was added in a 500 mL RBF and stirred. Acetic acid (0.14 mL, 2 mmol, 1 eq) and Grubbs 2nd Generation (0.21 g, 0.25 mmol, 0.1 eq) were added and stirred for 2 days in a closed system at 40 °C. The resulting mixture was evaporated and separated using column chromatography on silica gel (10-40% EtOAc in pentane) giving a brownish solid (0.91 mg, 1.47 mmol 62%). R_f = 0.15 (10% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2 H, *J* = 7.6 Hz, H$_{Fmoc}$), 7.60 (m, 2 H, H$_{Fmoc}$), 7.40 (t, 2 H, *J* = 7.2 Hz, H$_{Fmoc}$), 7.31 (t, 2 H, *J* = 7.6 Hz, H$_{Fmoc}$), 5.78 (m, 1 H, H-5), 5.52 (m, 2 H, H-4 and NH), 4.38 (d, 2 H, *J* = 7.2 Hz, CH$_{2\text{-}Fmoc}$), 4.24 (m, 2 H, H-3 and CH$_{Fmoc}$), 3.98 (d, 1 H, *J* = 10.0 Hz, H-1$_b$), 3.78 (d, 1 H, *J* = 10.0 Hz, H-1$_b$), 3.67 (m, 1 H, H-2), 3.26 (d, 1 H, *J* = 8.0 Hz, OH), 2.05 (m, 2 H, H-6), 1.43 (m, 2 H, H-7), 1.37-1.19 (m, 20 H, H-8 to H-17), 0.91 (s, 9 H, TBS$_{tBu}$), 0.87 (t, 3 H, *J* = 6.8 Hz, H-18), 0.08 (s, 6 H, TBS$_{Me}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.31 (C=O$_{Fmoc}$), 144.11, 144.01, 141.44 (x2) (4x C$_{q\text{-}Fmoc}$), 133.57 (C-5), 129.32 (C-4), 127.82, 127.17, 125.22, 124.90, 120.11 (CH$_{Fmoc}$), 74.64 (C-3), 67.16 (CH$_{2\text{-}Fmoc}$), 63.53 (C-1), 54.97 (C-2), 47.34 (CH$_{Fmoc}$), 32.44, 32.06, 29.83, 29.80, 29.77, 29.65, 29.50, 29.35, 29.33 (11x CH$_2$ C-6 to C-17), 25.95 (TBS$_{tBu}$), 22.83 (CH$_2$ C-6 to C-17), 18.26 (TBS$_{tBu\text{-}q}$), -5.47 (TBS$_{Me}$); IR (neat): 3441, 2924, 2852, 1716, 1463, 1263, 1089 cm^{-1}; HRMS calculated for $[C_{39}H_{61}NO_4Si + H]^+$: 636.4450, found 636.4452.

(9H-fluoren-9-yl)methyl ((2S,3R,E)-1-(tert-butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)oxy)octadec-4-en-2-yl)carbamate (22). Fully protected sphingosine **19** (50 mg, 0.09 mmol, 1 eq) was dissolved in dry DCM (0.5 mL), followed by addition of 1-pentadecene (47 µL, 0.17 mmol, 2 eq) . To the reaction was added acetic acid (5 µL, 0.09 mmol, 1 eq) and catalytic amount of Grubbs 2nd catalyst (7 mg, 0.009 mmol, 0.1 eq).



The reaction mixture was stirred at 40 °C in closed vessel for 2 days. The resulting mixture was concentrated and purified by silica column chromatography (5% EtOAc in pentane) giving a brownish solid **22** (12 mg, 0.016 mmol 18%). R_f = 0.75 (10% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, 2 H, *J* = 7.6 Hz, H$_{Fmoc}$), 7.57 (d, 2 H, *J* = 7.2 Hz, H$_{Fmoc}$), 7.38 (t, 2 H, *J* = 7.2 Hz, H$_{Fmoc}$), 7.29 (t, 2 H, *J* = 7.6 Hz, H$_{Fmoc}$), 7.21 (d, 2 H, *J* = 7.2 Hz, H$_{PMB}$), 6.88 (d, 2 H, *J* = 6.8 Hz, H$_{PMB}$), 5.71 (dt, 1 H, *J* = 12.4, 6.4 Hz, H-5), 5.44 (dd, 1 H, *J* = 15.6, 8.4 Hz, H-4), 5.06 (d, 1 H, *J* = 9.2 Hz, NH), 4.53 (d, 1 H, *J* = 11.2 Hz, CH$_{2\text{-}PMB\text{-}a}$), 4.30 (m, 2 H, CH$_{2\text{-}Fmoc}$), 4.24-4.19 (m, 2 H, CH$_{Fmoc}$ and CH$_{2\text{-}PMB\text{-}b}$), 3.92 (dd, 1 H, *J* = 10.0, 3.2 Hz, H-1$_a$), 3.86 (m, 1 H, H-3), 3.82-3.74 (m, 4 H, OMe$_{PMB}$ and H-2), 3.67 (dd, 1 H, *J* = 10.0, 4.0 Hz, H-1$_b$), 2.05 (m, 2 H, H-6), 1.34 (m, 2 H, H-7), 1.30-1.20 (m, 20 H, H-7 to H-17), 0.90 (s, 9 H, TBS$_{tBu}$), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18), 0.06 (s, 3 H, TBS$_{Me}$), 0.05 (s, 3 H, TBS$_{Me}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.04 (C=O$_{Fmoc}$), 144.21, 144.05, 141.38, 141.36 (4x C$_{q\text{-}Fmoc}$), 136.98 (C-5), 130.63 (C$_{q\text{-}PMB}$), 129.84, 127.73 (2x CH$_{Fmoc}$), 127.57 (C-4) 127.08, 1250.26 (2x CH$_{Fmoc}$), 120.05, 113.75 (2x CH$_{PMB}$), 79.09 (C-3), 70.00 (CH$_{2\text{-}PMB}$), 66.92 (CH$_{2\text{-}Fmoc}$), 61.64 (C-1), 55.54 (C-2), 55.34 (OMe$_{PMB}$), 47.31 (CH$_{Fmoc}$), 32.46 (C-6), 32.05, 30.40, 29.81, 29.78, 29.61, 29.49, 29.37, 29.34, 28.01, (10x CH$_2$ C-7 to C-17), 25.97 (TBS$_{tBu}$), 18.38 (TBS$_{tBu\text{-}q}$), 14.26 (C-18), -5.27 (TBS$_{Me}$), -5.35 (TBS$_{Me}$); IR (neat): 3444, 2924, 2852, 1726, 1450, 1264, 1082, 1056, 1037 cm^{-1}; HRMS calculated for $[C_{47}H_{69}NO_5Si + H]^+$; 765.5025, found 765.5023.

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



Fmoc-protected sphingosine **20** (1.95 g, 4.25 mmol, 1 eq) was dissolved in 20 mL DCM under protected atmosphere. 1-Pentadecene (2.3 mL, 8.5 mmol, 2 eq) and acetic acid (0.24 mL, 4.25 mmol, 1 eq) were added before addition of Grubbs 2nd catalyst (370 mg, 0.4 mmol, 0.1 eq). The mixture was stirred for 2 days at 40 °C. The reaction mixture was concentrated *in vacuo* and the crude residue was purified with silica gel chromatography (0-15% EtOAc in pentane) giving a white solid **7** (1.77 g, 2.76 mmol, 65%). R_f = 0.31 (30% EtOAc

in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2 H, *J* = 7.7 Hz, H_{Fmoc}), 7.63 – 7.55 (m, 2 H, H_{Fmoc}), 7.40 (t, 2 H, *J* = 8.2 Hz, H_{Fmoc}), 7.31 (t, 2 H, *J* 8.4 Hz, H_{Fmoc}), 7.20 (md, 2 H, *J* = 8.4 Hz, H_{PMB}), 6.84 (d, 2 H, *J* = 8.4 Hz, H_{PMB}), 5.78 (m, 1 H, H-5), 5.55 (d, 1H, *J* = 8.3 Hz, N-H), 5.46 (d, 1 H, *J* 15.4, 6.5, Hz, H-4), 4.56 (d, 1 H, *J* = 11.4 Hz, $CH_{2\text{-}PMB\text{-}a}$), 4.34 (m, 2 H, $CH_{2\text{-}Fmoc}$), 4.24-4.18 (m, 2 H, CH_{Fmoc} and $CH_{2\text{-}PMB\text{-}b}$), 4.07-3.97 (m, 2 H, H-$1_a$ and H-3), 3.74 (s, 3 H, OMe_{PMB}), 3.71 – 3.60 (m, 2 H, H-1_b and H-2), 2.08 (m, 2H, H6), 1.26-1.14 (m, 22 H, H-7 to H-17), 0.88 (t, 3 H, *J* = 6.7 Hz, H-18); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.5 ($C{=}O_{Fmoc}$), 144.08, 141.45 (4x $C_{q\text{-}Fmoc}$), 137.16 (C-5), 129.88 (CH_{PMB}), 127.83, 127.19 (2x CH_{Fmoc}), 125.23 (C-4), 125.29, 120.08 (2x CH_{Fmoc}) 114.03 (CH_{PMB}), 81.61 (C-3), 70.44 ($CH_{2\text{-}PMB}$), 66.95 ($CH_{2\text{-}Fmoc}$), 62.19 (C-1), 55.36 (OMe_{PMB}), 55.35 (C-2) 47.35 (CH_{Fmoc}), 32.46 (C-6), 32.05, 29.83, 29.81, 29.79, 29.76, 29.70, 29.58, 29.49, 29.43, 29.32, 29.22, 22.82 (11x CH_2, C-7 to C-17), 14.26 (C-18); IR (neat): 3464 and 3325, 2924, 2854, 1689, 1249, 1233 cm^{-1}; HRMS calculated for $[C_{41}H_{55}NO_5 + H]^+$: 642.4160, found 642.4157.

Glucosylsphingosine (23a). See general procedure for the glycosylation of protected sphingosine **7** with trichloro-imidate donor **5a**. Yield (0.1 mg 0.082 mmol, 82%). R_f = 0.4 (20% EtOAc in pentane); ^{1} H NMR (400 MHz, $CDCl_3$) δ 8.05 – 7.70 (m, 10 H, H_{arom}), 7.56 –7.16 (m, 20 H, H_{arom}), 6.81 (d, 2 H, *J* = 8.6 Hz, H_{PMB}), 5.93 (t, 1 H, *J* =, 10.0 Hz, H-3'), 5.71 (t, 1 H, *J* = 10.0 Hz, H-4'), 5.54 (t, 1 H, *J* = 8.0 Hz, H-2'), 5.45 (m, 1 H, H-5_{sp}), 5.29 (m, 1 H, H-4_{sp}), 4.89 (d, 1 H, *J* = 8.8 Hz, NH_{sp}), 4.78 (d, 1 H, *J* = 8.0 Hz, H-1'), 4.64 (dd, 1 H, *J* = 8.4, 2.8 Hz, $CH_{2\text{-}PMB\text{-}a}$), 4.52 (m, 1 H, $CH_{2\text{-}PMB\text{-}b}$), 4.43 (d, 1 H, *J* = 11.2 Hz, $CH_{2\text{-}Fmoc\text{-}a}$), 4.36-4.24, (m, 3 H, H-$1_{sp\text{-}a}$, $CH_{2\text{-}Fmoc\text{-}b}$ and H-6'$_a$), 4.15-4.04 (m, 2 H, H-5' and H-6'$_b$), 3.83 (m, 1 H, H-2_{sp}) 3.75-3.72 (m, 4 H, H-3_{sp} and OMe_{PMB}),3.69 (m, 1 H, H-$1_{sp\text{-}b}$), 1.92 (m, 2 H, H-6_{sp}), 1.35-1.19 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18_{sp}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 165.9, 165.4, 165,3 (4x $C{=}0_{Bz}$), 155.89 ($C{=}O_{Fmoc}$), 144.15, 144.00 141.42, 141.36 (4x $C_{q\text{-}Fmoc}$), 137.36 (C-5_{sp}), 133.61, 133.45, 133.40, 133.25 (4x CH_{arom}), 130.47 ($C_{q\text{-}arom}$), 129.96, 129.94, 129.88, 129.85, 129.64, 129.61, 129.50, 129.11, 128.88, 128.57, 128.57, 128.50, 128.45, 127.81, 127.79, 127,16 (CH_{arom} and $C_{q\text{-}arom}$), 125.23 (C-4_{sp}), 120.09 (CH_{Fmoc}), 113.84 (CH_{PMB}), 101.69 (C-1'), 79.08 (C-3_{sp}), 72.82 (C-3'), 72.37 (C-2' and C-5'), 70.32 ($CH_{2\text{-}PMB}$), 69.81 (C-4'), 68.55 (C-1_{sp}), 66.67 (C-6'), 63.28 ($CH_{2\text{-}Fmoc}$), 55.33 (OMe_{PMB}), 53.86 (C-2_{sp}), 47.33 (CH_{Fmoc}), 32.05 (C-6_{sp}), 29.84, 29.81, 29.79, 29.58, 29.49, 29.33, 29.28, 22.82, 22.82 (11x CH_2, C-7_{sp} to C-17_{sp}), 14.27 (C-18_{sp}); IR (neat): 2925, 2856, 1721, 1690, 1250, 1231, 1089, 1066, 1025 cm^{-1}; HRMS calculated for $[C_{75}H_{81}NO_{14} + H]^+$: 1220.5665, found 1220.5669.

[1,2,3,4,5,6-$^{13}C_6$]-Glucosylsphingosine (23b). See general procedure for the glycosylation of protected sphingosine **7** with trichloro-imidate donor **5b**. Yield (51 mg 0.041 mmol, 82%). R_f = 0.4 (20% EtOAc in pentane); ^{1}H NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$) δ 8.05 – 7.80 (m, 10 H, H_{arom}), 7.59 – 7.22 (m, 20 H, H_{arom}), 6.84 (d, 2 H, *J* = 8.4 Hz, H_{PMB}), 5.93 (m, 1 H, H-3'), 5.71 (m, 1 H, H-4'), 5.54 (m, 1 H, H-2'), 5.45 (m, 1 H, H-5_{sp}), 5.29 (m, 1 H, H-4_{sp}), 4.93 (d, 1 H, *J* = 8.8 Hz, NH_{sp}), 4.78 (m, 1 H, H-1'), 4.64 (m, 1 H, $CH_{2\text{-}PMB\text{-}a}$), 4.52 (m, 1 H, $CH_{2\text{-}PMB\text{-}b}$), 4.48 (d, 1 H, *J* = 11.2 Hz, $CH_{2\text{-}Fmoc\text{-}a}$), 4.36-4.28, (m, 3 H, H-$1_{sp\text{-}a}$, $CH_{2\text{-}Fmoc\text{-}b}$ and H-6'$_a$), 4.13-4.08 (m, 2 H, H-5' and H-6'$_b$), 3.83 (m, 1 H, H-2_{sp}) 3.75-3.72 (m, 4 H, H-3_{sp} and OMe_{PMB}),3.69 (m, 1 H, H-$1_{sp\text{-}b}$), 1.92 (m, 2 H, H-6_{sp}), 1.35-1.19 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18_{sp}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.12, 165.84, 165.36, 165.16 (4x $C{=}0_{Bz}$),), 155.87 ($C{=}O_{Fmoc}$), 144.03, 143.86 141.34, 141.15 (4x $C_{q\text{-}Fmoc}$), 137.04 (C-5_{sp}), 133.59, 133.44, 133.38, 133.23 (4x CH_{arom}), 130.44, 129.93, 129.86, 129.83, 129.58, 129.48, 128.90, 128.86, 128.64, 128.55, 128.48, 128.43, 127.80, 127.77, 127.14 (CH_{arom} and $C_{q\text{-}arom}$), 125.21 (C-4_{sp}), 120.07 (CH_{Fmoc}), 113.82 (CH_{PMB}), 101.40 (d, *J* = 41.0 Hz, C-1), 78.94 (C-3_{Sp}), 74.22-71.83 (m, C-2' and C-5'), 70.82-68.38 (m, C-4', $CH_{2\text{-}PMB}$, C-1_{sp}), 63.4 (d, *J* = 45 Hz, C-6'), 60.51 ($CH_{2\text{-}Fmoc}$), 55.31 (OMe_{PMB}), 54.34 (C-2_{Sp}), 47.30 ($CH_{2\text{-}Fmoc}$), 32.03 (C-6_{sp}), 29.79, 29.77, 29.57, 29.47, 29.31, 29.26, 22.80, 21.16 (C-7_{sp} to C-17_{sp}), 14.30 (C-18_{sp}); IR (neat): 2925, 2856, 1721, 1690, 1250, 1231, 1089, 1066, 1025 cm^{-1}; HRMS calculated for $[C_{69}{}^{13}C_6H_{81}NO_{14} + H]^+$: 1226.5239, found 1226.5252.

Glucosylsphingosine (25a). General procedure for deprotection of protected glycosylsphingosine. Yield (30 mg, 0.06 mmol, 80%). $[\alpha]_D^{22}$: -5.0 (c = 0.1 MeOH); ^{1}H NMR (600 MHz, MeOD-*d*$_4$) δ 5.87 (dtd, 1 H, *J* = 15.0, 6.8, 1.2 Hz, H-5$_{sp}$), 5.48 (ddt, 1 H, *J* = 15.4, 6.9, 1.5 Hz, H-4$_{sp}$), 4.33-4.29 (m, 2 H, H-1 and H-3$_{sp}$), 3.97-3.88 (m, 3 H, H-6 and H-1$_{a\text{-}sp}$), 3.66 (m, 1 H, H-1$_{b\text{-}sp}$), 3.40-3.32 (m, 2 H, H-5 and H-2$_{sp}$), 3.29-2.21 (m 3 H, H-2, H-3 and H-4), 2.1 (q, 2 H, *J* = 7.2 Hz, H-6$_{sp}$), 1.42 (m, 2 H, H-7$_{sp}$), 1.36-1.22 (m, 20 H, H-8$_{sp}$ to H-17$_{sp}$), 0.9 (t, 3 H, *J* = 7.0 Hz, H-18$_{sp}$); ^{13}C NMR: (151 MHz, MeD-*d*$_4$) δ 136.8 (C-5$_{sp}$), 128.4 (C-4$_{sp}$), 104.1 (C-1), 78.1 (C-4), 77.9 (C-5), 74.8 (C-3), 71.5 (C-2), 70.9 (C-3$_{sp}$), 67.3 (C-6), 62.5 (C-1$_{sp}$), 56.8 (C-2$_{sp}$), 33.4 (C-6$_{sp}$), 33.1, 30.82, 30.81 (2x), 30.78, 30.77, 30.66, 30.50, 30.41, 30.18, 23.6 (11x CH$_{2\text{-}Sp}$), 14.2 (C-18$_{Sp}$); IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm^{-1}; HRMS calculated for $[C_{24}H_{47}NO_7 + H]^+$: 462.3431, found 462.3424.

[1,2,3,4,5,6-$^{13}C_6$]-Glucosylsphingosine (25b). General procedure for deprotection of protected glycosylsphingosine. Yield (15 mg, 0.03 mmol, 78%). $[\alpha]_D^{22}$: -4.8 (c = 0.1 MeOH); ^{1}H NMR (^{13}C-decoupled, 400 MHz, MeOD-*d*$_4$) δ 5.87 (m, 1 H, H-5$_{sp}$), 5.52 (m, 1 H, H-4$_{sp}$), 4.31 (m, 2 H, H-1 and H-3$_{sp}$), 4.05-3.82 (m, 3 H, H-6, H-1$_{a\text{-}Sp}$), 3.69 (m 1 H, H-1$_{b\text{-}Sp}$), 3.56-3.39 –(m, 2 H, H-5 and H-2$_{sp}$), 3.31-3.10 (m, 3 H, H-2, H-3, H-4), 2.10 (m, 2 H, H-6$_{sp}$), 1.42 (m, 2 H, H-7$_{sp}$), 1.38-1.22 (m, 20 H, H-8$_{sp}$ to H-17$_{sp}$), 0.88 (m, 3 H, H-18$_{sp}$); ^{13}C NMR (101 MHz, MeOD-*d*$_4$) δ 131.4 (C-5$_{Sp}$), 128.7 (C-4$_{Sp}$), 104.1 (d, *J* = 46.0 Hz, C-1), 78.4-76.04 (m, C-4 and C-5), 74.7 (dd, *J* = 52.0, 45.0 Hz, C-3), 71.5 (m, C-2 and C-3$_{sp}$), 67.3 (d, *J* = 43.0 Hz, C-6), 62.5 (C-1$_{sp}$), 56.8 (C-2$_{sp}$), 33.0 (C-6$_{sp}$), 30.75, 30.72 (3x), 30.78, 30.77, 30.60, 30.50, 30.43, 30.36, 23.7 (11x CH$_{2\text{-}Sp}$), 14.4 (C-18$_{Sp}$) IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm^{-1}; IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm^{-1}: HRMS calculated for $[C_{18}{}^{13}C_6H_{47}NO_7 + H]^+$: 468.3005, found 468.2996.

6-deoxy-6-Azido-Glucosesphingosine (27). See general procedure for the glycosylation of protected sphingosine **7** with trichloro-imidate donor **26**. Yield (0.18 g, 0.16 mmol, 79%) R$_f$ = 0.55 (20% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95-7.89 (m 4 H, H$_{arom}$), 7.85-7.76 (m, 4 H, H$_{arom}$), 7.55-7.50 (m, 2 H, H$_{arom}$), 7.45 (m, 15 H, H$_{arom}$), 6.87 (d, 2 H, *J* = 8.8 Hz, H$_{PMB}$), 5.90 (t, 1 H, *J* = 10.4 Hz, H-3'), 5.53-5.44 (m, 3 H, H-5$_{sp}$, H-2' and H-4'), 5.29 (dd, 1 H, *J* = 15.2, 7.6 Hz, H-4$_{sp}$), 4.86 (d, 1 H, *J* = 8.8 Hz, NH$_{sp}$), 4.76 (d, 1 H, *J* = 8.0 Hz, H-1'), 4.47 (d, 1 H, *J* = 11.2 Hz, CH$_{2\text{-}PMB\text{-}a}$), 4.32-4.25 (m, 3 H, CH$_{2\text{-}PMB\text{-}b}$, H-1$_{sp\text{-}a}$, CH$_{2\text{-}Fmoc\text{-}a}$), 4.10-4.05 (m, 2 H, CH$_{2\text{-}Fmoc\text{-}b}$ and CH$_{Fmoc}$), 3.94 (m, 1 H, H-5'), 3.85-3.70 (m, 6 H, OMe$_{PMB}$, H-1$_{sp\text{-}b}$, H-2$_{sp}$ and H-3$_{sp}$), 3.53 (dd, *J* = 13.6, 8.8 Hz, H-6$_a$'), 3.38 (d, 1 H, *J* = 12.0 Hz, H-6$_b$'), 1.90 (m, 2 H, H-6$_{sp}$), 1.33-1.19 (m, 22 H, H-7$_{sp}$ to H-17$_{sp}$), 0.88 (t, 3 H, *J* = 6.8 Hz, H-18$_{sp}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.82, 165.43, 165.27 (3x C=O$_{OBz}$), 159.19 (C=O$_{Fmoc}$), 144.16, 144.00, 141.42, 141.37 (4x C$_{q\text{-}Fmoc}$), 137.39 (C-5$_{sp}$), 133.81, 133.44 (CH$_{arom}$), 130.54 (C$_{q\text{-}arom}$), 129.98, 129.86, 129.69, 129.51, 129.04, 128.85, 128.66, 128.55, 128.45, 127.80, 127.15 (CH$_{arom}$ and C$_{q\text{-}arom}$), 125.30 (C-4$_{sp}$), 120.11, 120.08 (2x CH$_{Fmoc}$), 113.90, 113.83 (2x CH$_{PMB}$), 101.39 (C-1'), 79.10 (C-3$_{sp}$), 74.12 (C-5'), 72.59 (C-3'), 72.14 (C-2'), 70.14 (C-4'), 70.15 (CH$_{2\text{-}PMB}$), 68.44 (C-1$_{sp}$), 66.61 (CH$_{2\text{-}Fmoc}$), 55.37 (OMe$_{PMB}$), 53.83 (C-2$_{sp}$), 51.34 (C-6'), 47.33 (CH$_{Fmoc}$), 32.34 (C-6$_{sp}$), 29.83, 29.81, 29.78, 29.57, 29.49, 29.33, 29.26, 27.83, 22.82 (11x CH$_2$ C-7$_{sp}$ to C-17$_{sp}$), 14.27 (C-18$_{sp}$);); IR (neat): 2925, 2856, 2104, 1721, 1690, 1250, 1231, 1089, 1066, 1025 cm^{-1}; HRMS calculated for $[C_{68}H_{76}N_4O_{12} + H]^+$: 1141.5468, found 1141.5464.

6-deoxy-6-Azido-Glucosesphingosine (28). General procedure for deprotection of protected glycosylsphingosine. Yield (25 mg, 0.06 mmol, 77%). ^{1}H NMR (400 MHz, MeOD-*d*$_4$) δ 5.84 (dt, 1 H, *J* = 15.2, 7.6 Hz, H-5$_{sp}$), 5.48 (dd, 1 H, *J* = 15.2, 6.8 Hz, H-4$_{sp}$), 4.36 (d, 1 H, *J* = 8.0 Hz, H-1'), 4.28 (t, 1 H, *J* = 5.6 Hz, H-3$_{sp}$), 3.99 (m, 1 H, H-1$_{a\text{-}sp}$), 3.82 (d, 1 H, *J* = 10.8 Hz, H-1$_{b\text{-}sp}$), 3.54-3.24 (m, 7 H, H-2$_{sp}$, H-2', H-3', H-4' and H-5', H-6'), 3.08 (m, 2 H, H-6$_{sp}$), 1.41 (m, 2 H, H-7$_{sp}$), 1.36-1.28 (m, 20 H, H-8$_{sp}$ to H-17$_{sp}$), 0.90 (t, 3 H, *J* = 6.4 Hz, H-18$_{sp}$); ^{13}C NMR (101 MHz, MeOD-*d*$_4$) δ 136.56 (C-5$_{sp}$), 128.57 (C-4$_{sp}$), 103.86 (C-1'), 77.54, 77.13, 74.83, 72.25 (C-2', C-3', C-4', C-5'), 71.36 (C-3$_{sp}$), 67.37 (C-1$_{sp}$), 56.27 (C-2$_{sp}$), 52.65 (C-6'), 33.07 (C-6$_{sp}$),

30.79, 30.76, 30.63, 30.47, 30.39, 30.16, 23.73 (11x CH_2 C-7_{sp} to C-17_{sp}), 14.45 (C-18_{sp}); IR (neat): 3300, 2917, 2850 , 2100, 1668, 1434, 1201, 800, 721 cm^{-1}; HRMS calculated for $[C_{24}H_{46}N_4O_6 + H]^+$: 487.3497, found 487.3506.

Galactosylsphingosine (30). See general procedure for the glycosylation of protected sphingosine **7** with trichloro-imidate donor **29**. Yield (91 mg 0.075 mmol, 75%). R_f = 0.4 (20% EtOAc in pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, 2 H, *J* = 7.6 Hz, $H_{arom\text{-}OBz}$), 8.02 (d, 2 H, *J* = 7.2 Hz, $H_{arom\text{-}OBz}$), 7.99 (d, 2 H, *J* = 8.0 Hz, $H_{arom\text{-}OBz}$), 7.81-7.75 (m, 4 H, H_{arom}), 7.61 (t, 1 H, *J* = 7.6 Hz, H_{arom}), 7.55-7.18 (m, 19 H, H_{arom}), 6.83 (d, 2 H, *J* = 8.8 Hz, H_{PMB}), 6.01 (m, 1 H, H-4'), 5.77 (dd, 1 H, *J* = 10.4, 10.0 Hz, H-2'), 5.62 (dd, 1 H, *J* = 10.4, 3.2 Hz, H-3'), 5.44 (dt, 1 H, *J* = 15.6, 6.4 Hz, H-5_{sp}), 5.27 (dd, 1 H, *J* = 16.4, 8.0 Hz, H-4_{sp}), 4.90 (d, 1 H, *J* = 8.8 Hz, NH_{sp}), 4.75 (d, 1 H, *J* = 7.6 Hz, H-1'), 4.65 (dd, 1 H, *J* = 11.2, 6.4 Hz, $CH_{2\text{-}PMB}$), 4.48-4.43 (m, 2 H, $CH_{2\text{-}PMB}$, $CH_{2\text{-}Fmoc\text{-}a}$), 4.37-4.27 (m, 4 H, $CH_{2\text{-}Fmoc\text{-}b}$, H-$1_{sp\text{-}a}$, H-5' and H-$6_a$), 4.12-4.05 (m, 2 H, H-$6_b$' and CH_{Fmoc}), 3.83-3.70 (m, 6 H, OMe_{PMB}, H-$1_{sp\text{-}b}$, H-2_{sp}, H-3_{sp}), 1.90 (m, 2 H, H-6_{sp}), 1.37-1.20 (m, 22 H, H-7_{sp} to H-17_{sp}), 0.87 (t, 3 H, *J* = 7.2 Hz, H-18_{sp}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.15, 2x 165.64, 165.47 (4x $C{=}O_{Bz}$), 159.21 ($C{=}0_{Fmoc}$), 144.13, 143.95, 141.42, 141.37 ($C_{q\text{-}Fmoc}$), 137.32 (C-5_{sp}), 133.71, 133.43 (CH_{arom}), 130.50 ($C_{q\text{-}arom}$), 130.12, 129.89, 129.86, 129.42, 129.14, 128.88, 128.77, 128.61, 128.58, 128.42, 127.79, 127.15 (CH_{arom} and $C_{q\text{-}arom}$), 125.16 (C-4_{sp}), 120.08 (CH_{Fmoc}), 113.85 (CH_{PMB}), 102.04 (C-1'), 79.09 (C-3_{sp}), 71.63 (C-3'), 71.48 (C-5'), 70.28 ($CH_{2\text{-}PMB}$), 70.20 (C-2_{sp}), 68.61 (C-1_{sp}), 68.20 (C-4'), 66.54 (C-6'), 62.14 ($CH_{2\text{-}Fmoc}$), 55.31 (OMe_{PMB}), 53.97 (C-2_{sp}), 47.36 (CH_{Fmoc}), 32.35 (C-6_{sp}), 29.82, 29.78, 29.58, 29.33, 29.28, 22.82 (11x CH_2 C-7_{sp} to C-17_{sp}), 14.26 (C-18_{sp}). IR (neat): 2924, 2854, 1722, 1689, 1249, 1233, 1091, 1066, 1026 cm^{-1}; HRMS calculated for $[C_{75}H_{81}NO_{14} + H]^+$: 1220.5665, found 1220.5671.

Galactosylsphingosine (31). General procedure for deprotection of protected glycosylsphingosine. Yield (25 mg, 0.55 mmol, 74%). ^{1}H NMR (400 MHz, MeOD-d_4) δ 5.97 (dd, 1 H, *J* = 15.6, 6.6 Hz, H-5), 5.69 (dd, 1 H, *J* = 15.4, 6.9 Hz, H-4), 4.32 (d, 1 H, *J* = 7.6 Hz, H-1'), 4.09 (m, 1 H, H-3), 3.96 (t, 1 H, *J* = 6.4 Hz, H-5) 3.89-3.86 (m, 2 H, H-6'), 3.84-3.81 (m, 1 H, H-1_a), 3.77-3.70 (m, 2 H, H-1_b and H-2), 3.58- 3.48 (m, 3 H, H-2', H-3' and H-4'), 2.05 (m, 2 H, H-6), 1.48 (m, 2 H, H-7), 1.33-1.23 (m, 20 H, H-8 to H-17), 0.90 (t, 3 H, *J* = 6.8 Hz, H-18); ^{13}C NMR (101 MHz, MeOD-d_4) δ 141.73 (C-5), 123.06 (C-4), 104.42 (C-1'), 77.00 (C-4'), 74.72 (C-3'), 72.46 (C-2'), 70.67 (C-6'), 70.34 (C-3), 62.67 (C-1), 54.07 (C-2), 33.07 (C-6), 30.79, 30.76, 30.47, 26.52, 23.73 (11x CH_2 C-7 to C-17), 14.44 (C-18); IR (neat): 3300, 2918, 2850, 1668, 1435, 1202, 1134, 1074, 1026, 800, 721 cm^{-1}; HRMS calculated for $[C_{24}H_{47}NO_7 + H]^+$: 462.3431, found 461.3425.

3.4 References

[1] P. Wisse, H. Gold, M. Mirzaian, M. J. Ferraz, G. Lutteke, R. J. B. H. N. van den Berg, H. van den Elst, J. Lugtenburg, G. A. van der Marel, J. M. F. G. Aerts, J. D. C. Codée, H. S. Overkleeft, *Eur. J. Org. Chem.* **2015,** *12*, 2661-2677.

[2] H. Gold, R. G. Boot, J. M. F. G. Aerts, H. S. Overkleeft J. D. C. Codée, G. A. van der Marel, *Eur. J. Org. Chem.* **2011**, *9*, 1652-1663.

[3] a) R. Bommer, R. R. Schmidt, *Liebigs Ann. Chem*. **1989**, 1107-1111. b) K. C. Nicolaou, T. J. Caulfield, H. Katoaka, N. A. Stylianides, *J. Am. Chem. Soc.* **1990**, *112*, 3693-3695. c) M. Wilstermann, G. Magnusson, *J. Org. Chem.* **1997**, *62*, 7961-7971.

[4] S. Kim, S. Lee, T. Lee, H. Ko, D. Kim, *J. Org. Chem.* **2006**, *71*, 8661-8664.

[5] A. N. Rai, A. Basu, *Org. Lett.* **2004**, *6*, 2861-2863.

[6] A. N. Rai, A. Basu, *J. Org. Chem.* **2005**, *70*, 8228-8230.

[7] T. Yamamoto, H. Hasegawa, T. Hakogi, S. Katsumura, *Org. Lett.* **2008**, *8,* 5569-5572.

[8] S. Nahm, S. M. Weinreb, *Tetrahedron Lett.* **1981**, *22*, 3815-3818.

[9] A. Gomtsyan, *Org. Lett.* **2000**, *2,* 1999-2002.

[10] R. V. Hoffman, N. Maslouh, F. Cervantes-Lee, *J. Org. Chem.* **2002**, *67*, 1045-1056.

[11] a) N. Barroca-Aubry, M. Benchekroun, F. Gomes, D. Bonnaffe, *Tetrahedron Lett.* **2013**, *54*, 5118-5121. b) Y.-G. Wang, Y. Kobayashi, *Org. Lett.* **2002**, *26*, 4615-4618.

[12] A. K. Chatterjee, T.-L. Choi, D. P. Sanders, R. H. Grubbs, *J. Am. Chem. Soc.* **2003**, *125*, 11360-11370.

[13] Y. Lui, L. When, L. Li, M. R. Gadi, W. Guan, K. Huang, Z. Xiao, M. Wei, C. Ma, Q. Zhang, H. Yu, X. Chen, P. G. Wang, J. Fang, *Eur. J. Org. Chem.* **2016**, 4315-4320.

[14] a) A. R. Parameswar, J. A. Hawkins, L. K. Mydock, M. S. Sands, A. V. Demchenko, *Eur. J. Org. Chem.* **2010**, 3269-3274. b) N. Ding, W. Zhanf, G. Lv, Y. Li, *Arch. Pharm. Chem. Life Sci.* **2011**, *344*, 786-793.

[15] H. Gold, M. Mirzaian, N. Dekker, M. J. Ferraz. J. Lugtenburg, J. D. C. Codée, G. A. van der Marel, H.S. Overkleeft, G. E. Linthorst, J. E. M. Groener, J. M. F. G. Aerts, B. J. H. M. Poorthuis, *Clin. Chem.* **59**, *3*, 547-556.

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

[17] A. C. E. Graziano, V. Cardile, *Gene* **2015**, *555*, 2-13.