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Chapter 1
Chemical Tools to Study Sphingolipid Metabolism

1.1 Introduction

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

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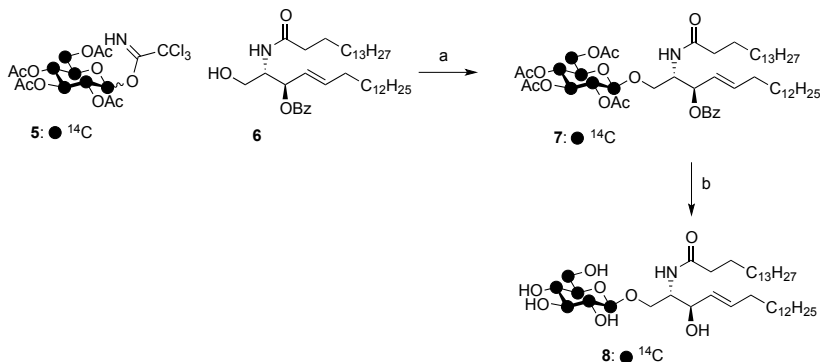
1.2 Radioisotope labeled sphingolipids

In the past decades, several strategies for the preparation of radio-isotopic sphingolipids have been reported. Carbon-14[2-6] and tritium (hydrogen-3)[7-17] are obvious choices with respect to the nature of the radioisotope employed and examples of both have appeared in the literature. The most commonly applied method for introducing carbon/hydrogen isotopes comprises *N*-acylation of sphingosine with an appropriately labeled fatty acid.[2-4, 7,20] Other commonly used methods to introduce radioisotopes are reductive tritiation of the alkene bond in sphingosine (catalytic palladium on charcoal, $^3H_2$ (g)[8,9], or by reducing the ketone of 3-ketosphingosine to sphingosine using sodium borotritide (NaB$^3H_4$).[10-13] Radioisotopes can also be introduced to the carbohydrate moiety in glycosphingolipids. For instance, introduction of tritium (^3H) in the glucose moiety of glucosylceramide can be accomplished by selective manipulation of the C6-hydroxyl of glucosylceramide **1** as described in Scheme 1.1. Selective 6-O-tritylation, followed by global O-acetylation and removal of the trityl group using acidic conditions resulted in partially protected resulted glucosylceramide **2**. The free primary hydroxyl in **2** was oxidized to aldehyde **3** and next reduced with NaB$^3H_4$ (**3** to **4**) followed by global deprotection, yielding tritium-labeled glucosylceramide **5**.[14] A similar strategy (selective oxidation followed by tritide reduction) has been developed for galactosyl/GM2/GalNAc[15,16] sphingolipids, although here the primary alcohol-to-aldehyde transformation was effected by the use of galactose oxidase.

Scheme 1.1 Synthesis of tritium-labeled glucosylceramide **5**.
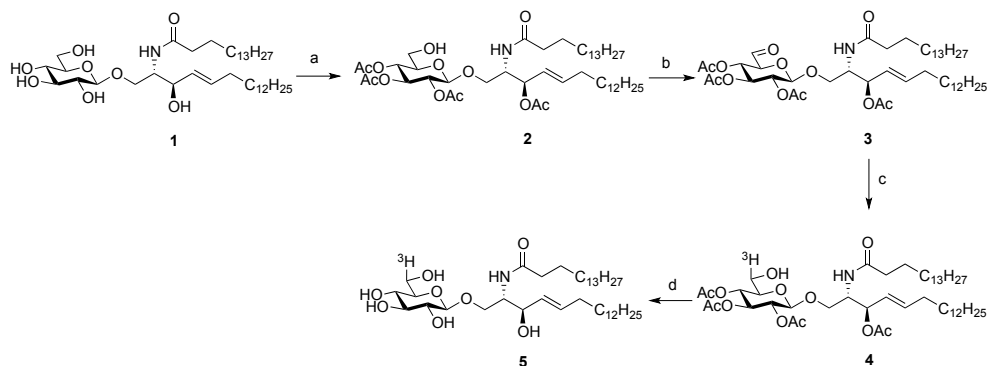
Reagents and conditions: (a) (i) TrCl, pyridine, 40 °C, 18 h; (ii) Ac₂O, pyridine, r.t., 20 h; (iii) HBr (33% in HOAc), HOAc/DCM (1:1), -10 °C, 5 min; (b) (i) *N,N*-dicyclohexylcarbodiimide, H₃PO₄, DMSO; (ii) HOAc; (c) (i) NaB³H₄, 1 mM NaOH (aq), THF, r.t., 20 h; (ii) 1 M HOAc (aq); (d) NaOMe, MeOH, r.t., 20 h.

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

Scheme 1.2 Synthesis of ¹⁴C-glucosylceramide.

Reagents and conditions: (a) BF₃OEt₂, DCM, -10 °C to r.t., 10 h, 55%; (b) NaOMe, MeOH, r.t., 24 h, 95%.

1.3 Stable isotope-labeled sphingolipids

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

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

Scheme 1.3. Synthesis of deuterium labeled sphingomyelin **11**.
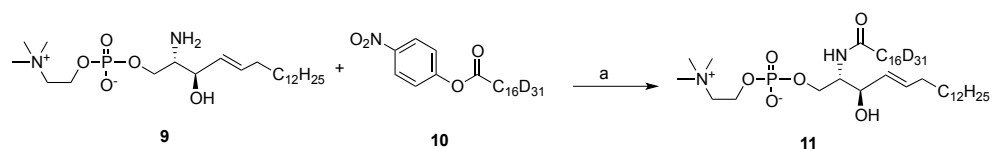
Reagents and conditions: (a) K_2CO_3, DMF/DCM, r.t., 24 h.

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

Scheme 1.4. Synthesis of $^{13}C_6$-labeled glucosylsphingosine **15**.

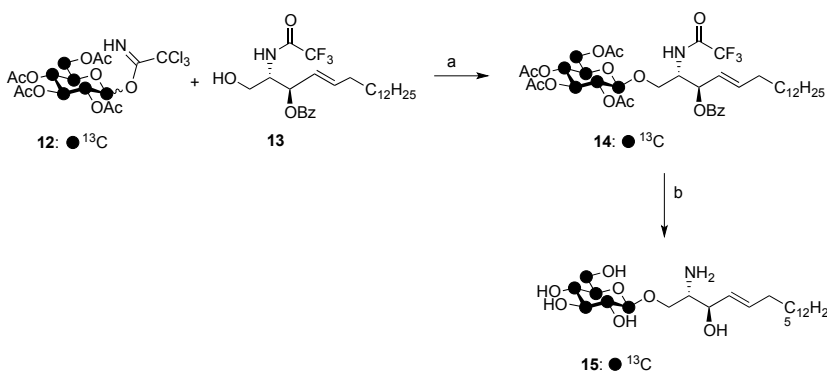Reagents and conditions: (a) BF_3OEt_2, DCM, -10 °C to r.t., 10 h, 27%; (b) NaOMe, MeOH, r.t., 24 h, 78%.

1.4 Fluorescent sphingolipids

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

Alternative fluorophores are borondipyrromethene (BODIPY),[26-31] pyrene,[32-35] diphenylhexatrientyl,[36] lissamine rhodamine,[37] Nile red[38] and dansyl.[39] Again, *N*-acylation of sphingosine with an appropriately modified fatty acid to give the corresponding ceramide has been the method of choice. As an example (Scheme 1.5), the synthesis of NBD-glucosylceramide **18** by *N*-acylation of glucosylsphingosine **16** with NBD-hexanoic acid **17** under the agency of PPh$_3$ and 2,2-dipyridyldisulfide has been accomplished.[22]

Scheme 1.5 *N*-acylation of glucosylsphingosine **16** with NBD-hexanoic acid **17**.
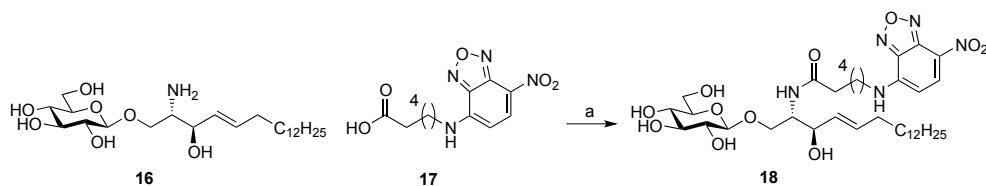
Reagents and conditions: (a) PPh$_3$, 2,2 dipyridyldisulfide, DCM.

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

Scheme 1.6 Synthesis of BODIPY-labeled stereoisomers of lactosylceramide **23**.
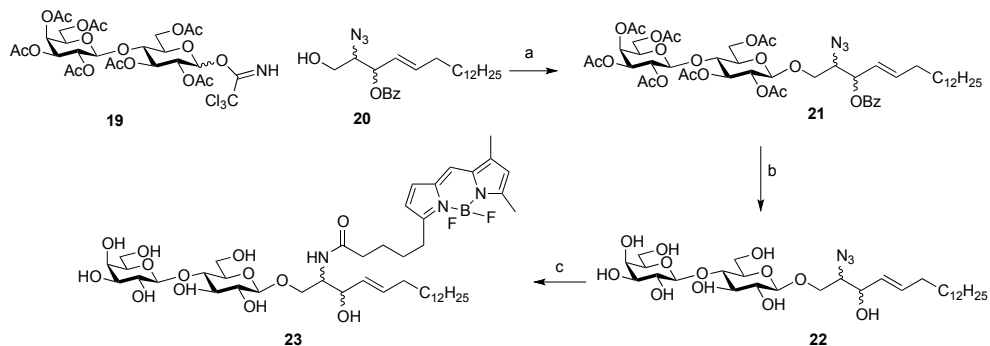
Reagents and conditions: (a) BF$_3$OEt$_2$, DCM, 20 h, (2*R*,3*R*) 66%, (2*R*,3*S*) 54%; (b) NaOMe, MeOH, 6 h, (2*R*,3*R*) 68%, (2*R*,3*S*) 62%; (c) BODIPY-C$_5$-NHS, PPh$_3$, THF/H$_2$O (9:1), (2*R*,3*R*) 38%, (2*R*,3*S*) 36%, (2*S*,3*S*) 38% (over three steps).

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

Scheme 1.7 Synthesis of NBD-labeled sphingosine-1-phospate **30**.
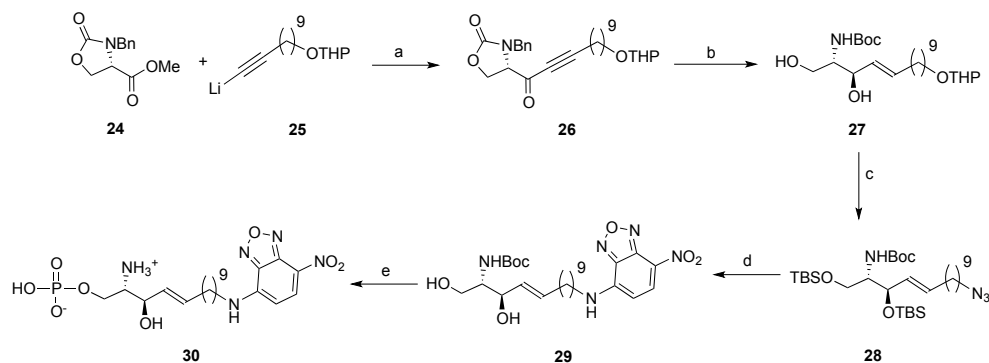
Reagents and conditions: (a) *n*-BuLi, THF, -100 ºC, 93% (b) (i) diisobutylaluminum hydride, 2,6-di-*tert*-butyl-4-methylphenoxide, toluene, 0 ºC, 99%; (ii) Li, NH$_3$, THF, reflux, 96%; (iii) KOH, EtOH, reflux; (iv) Boc$_2$O, K$_2$CO$_3$, THF, H$_2$O, 0 ºC, 84%; (c) (i) TBSCl, imidazole, DMF; (ii) MgBr$_2$, Et$_2$O, 82% (over the two steps); (iii) MsCl, Et$_3$N, THF; (iv) NaN$_3$, DMF, 50 ºC, 89% (for two steps); (d) (i) PPh$_3$, 10% aq THF, 60 ºC; (ii) NBDCl, Et$_3$N, THF; (iii) 2 M HCl, MeOH, 82% (over the three steps); (e) (i) CBr$_4$, P(OMe)$_3$, pyridine, -10 ºC, 82%; (ii) TMSBr, CH$_3$CN, 71%.

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

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

31 **32a** n = 4 **33a** n = 4 **34a** n = 4
32b n = 7 **33b** n = 7 **34b** n = 7
32c n = 9 **33c** n = 9 **34c** n = 9
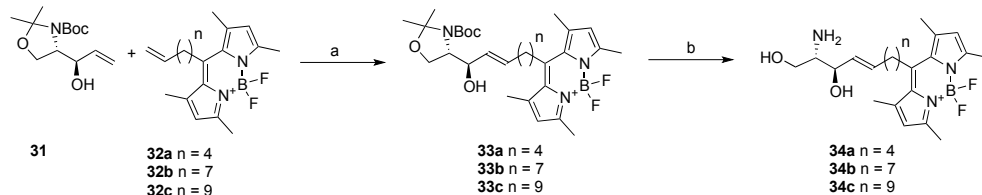
Reagents and conditions: (a) Grubbs' 2nd catalyst, DCM, reflux, **31a**: 67%, **31b**: 79%, **31c**: 57%; (b) HCl, dioxane, **31a**: 48%, **31b**: 68%, **31c**: 54%.

1.5 Fluorogenic ceramides

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

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

31 **35** **36** **37**
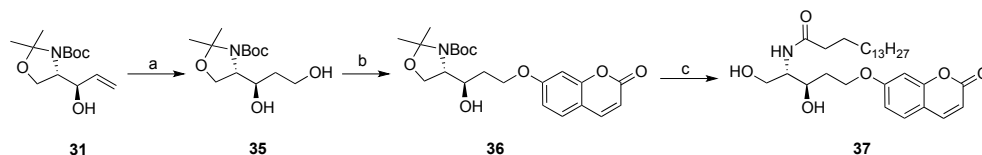
Reagents and conditions: (a) (i) BH$_3$, THF, 0 ^{o}C to r.t., 4 h; (ii) H$_2$O$_2$, 80%; (b) (i) MsCl, Et$_3$N, DCM, 0 ^{o}C, 30 min; (ii) umbelliferone, CsCO$_3$, acetone, 65 ^{o}C, 6 h; (c) (i) TFA, H$_2$O, DCM, 0 ^{o}C, 10 min; (ii) palmitoyl chloride, NaOAc, H$_2$O, DCM, 0 ^{o}C to r.t., 16 h.

1.6 Bioorthogonal sphingolipids.

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

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

**Scheme 1.10** Synthesis of azido-sphingosine **41** and azido-sphinganine **44**.
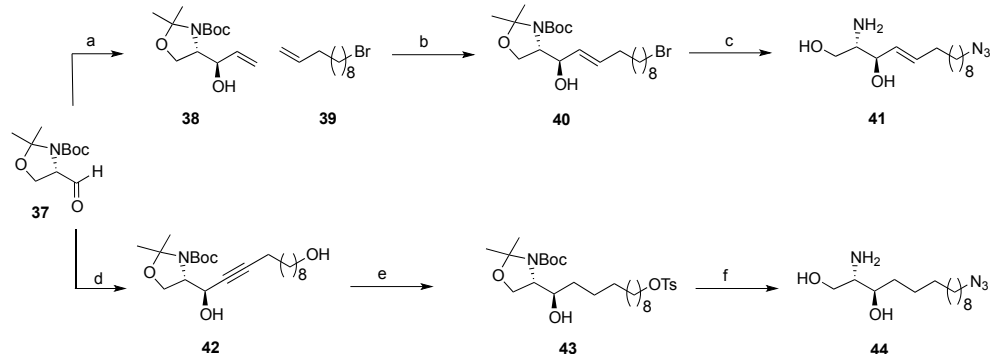
Reagents and conditions: (a) (i) Tetravinyl tin, THF, *n*-BuLi, -78 °C to r.t, 1h; (ii) **37**, THF, -78 °C to r.t., 20 h, 49%; (b) Grubbs' 2nd generation catalyst, DCM, reflux, 59%; (c) (i) NaN$_3$, DMF, 80 °C, 93%; (ii) HCl, MeOH, r.t., 1 h, 84%; (d), undec-10-yn-1-ol, BuLi, HMPA, THF, -78 °C, 50%, (e) (i) H$_2$ (g), Rh catalyst, MeOH, 89%; (ii) TsCl, DMAP, Et$_3$N, DCM, r.t., 58%; (f) (i) NaN$_3$, DMF, 80 °C, 76%; (ii) HCl, MeOH, r.t., 1 h, 85%.

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

**Scheme 1.11** Synthesis of *N*-alkyne glucosylceramide **48**.
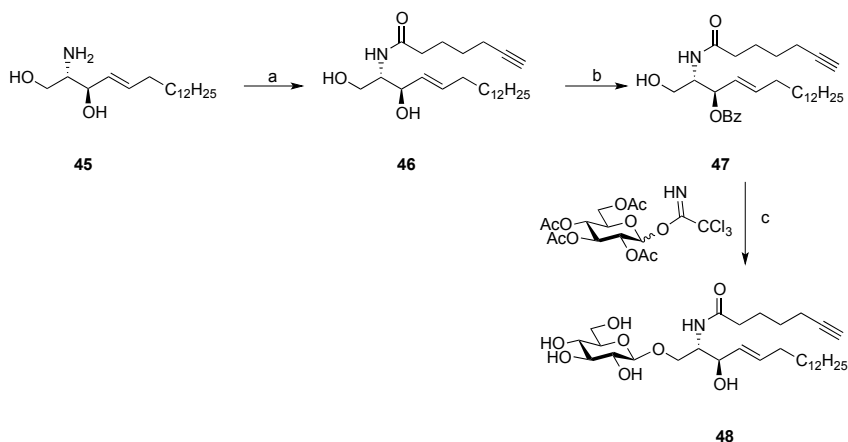
Reagents and conditions: (a) 7-heptynoic acid *N,O*-succinate ester, Et$_3$N, DCM, r.t., 16 h; (b) (i) TBDMSCl, imidazole, DCM, r.t., 20 h, 60% (two steps); (ii) BzCl, pyridine, r.t., 2 h, 95%, (iii) HF.pyridine, THF, r.t., 16 h, 50%; (c) (i) BF$_3$OEt$_2$, DCM, r.t., 2.5 h, 44%; (ii) NaOMe, MeOH, r.t. 16 h; 71%.

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

Scheme 1.12. Synthesis of 6-deoxy-6-azido glucosylceramide.
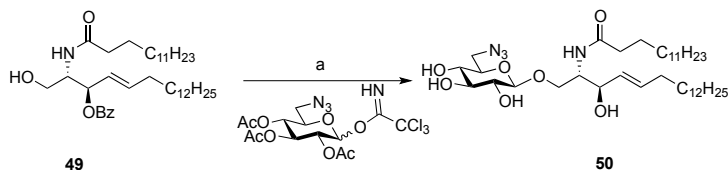
Reagents and conditions: (a) BF$_3$OEt$_2$, DCM, r.t., 2.5 h, 20%; (ii) NaOMe, MeOH, r.t. 16 h, 48%.

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

Scheme 1.13 Synthesis of alkyne-sphingomeylin **55**.
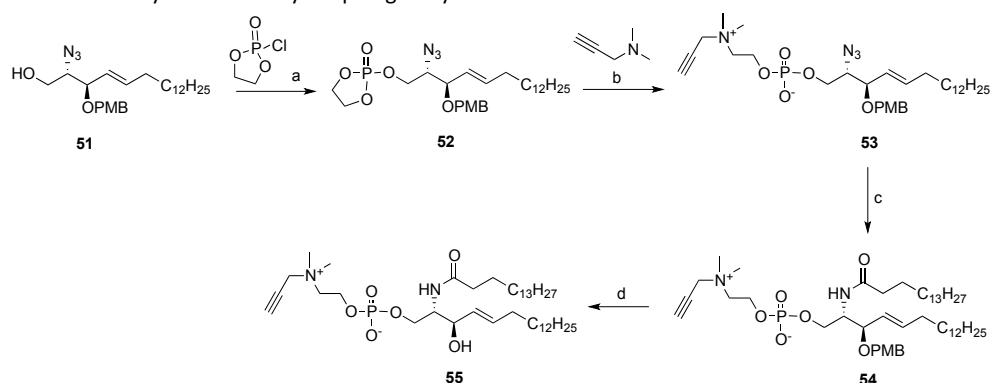
Reagents and conditions: (a) Et$_3$N, DMAP, toluene, 12 h; (b) TMSOTf, DCM, 12 h, 70% (for two steps); (c) (i) Zn, HOAc, 24 h; (ii) NHS-palmitate, DMAP (cat), DCM, 12 h; (d) TFA, DCM, 0 °C, 1 h, 76% (for two steps).

1.7 Photo-reactive sphingolipids

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

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

Scheme 1.14 Synthesis of *N*-acyl-[^{35}S]-diazirine ceramide **58**.
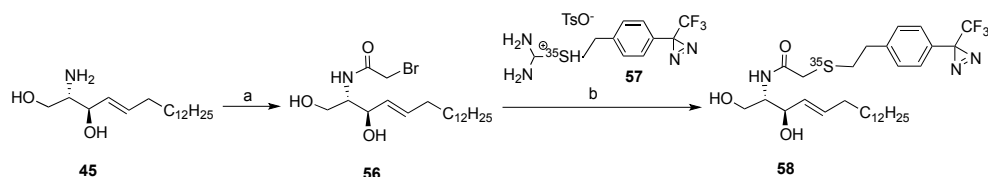
Reagents and conditions: (a) 2-bromoacetyl chloride, Et$_3$N, MeOH, -40 ^{o}C to r.t., 1 h, 91%; (b) **57**, K$_2$CO$_3$, MeOH, 55-70 ^{o}C; 39%.

An alternative sphingosine-based photo-cross linker, now with the diazirine embedded within the sphingosine backbone, rather than the fatty acid moiety, and bearing a tritium atom, is represented by compound **63**, the synthesis of which is depicted in Scheme 1.15. Ester **59** was reacted with dimethoxymethyl phosphonate forming ketophosphonate **60**.[48] Horner-Wadsworth-Emmons olefination of aldehyde **61** with ketophosphonate **60** provided protected 3-keto-sphingosine **62**, which was reduced with NaB^{3}H$_4$, followed by acidic removal of the isopropylidene and *N*-Boc protective groups yielding **63**. More recently, variation of this synthesis strategy enabled the synthesis of compound **66** (Scheme 1.15), now bearing a bioorthogonal alkyne instead of a radioisotope as a reporter entity.

Scheme 1.15 Synthesis of tritium-diazirine-sphingosine **63** and alkyne-diazirine-sphingosine **66**.
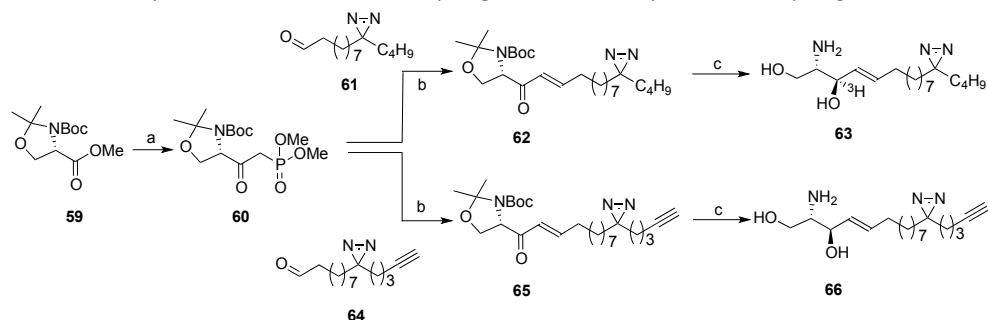
Reagents and conditions: (a) (i) CH$_3$PO(OMe)$_2$, BuLi, THF, -78 ^{o}C, 30 min; (ii) **59**, -78 ^{o}C, 30 min; (iii) 10% citric acid (aq); (b) (i) K$_2$CO$_3$, H$_2$O, CH$_3$CN, 5 min; (ii) **61** or **64**, DCM, 45 ^{o}C, 6 h; (c) (i) NaBH$_4$/NaB^{3}H$_4$, CeCl$_3$. H$_2$O, MeOH, 4 ^{o}C, 3 h; (ii) 6 M HCl, MeOH, 50 ^{o}C, 6 h.

1.8 Fluorine-containing sphingolipids

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

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

Scheme 1.16 Synthesis of fluorine-containing sphingolipids.
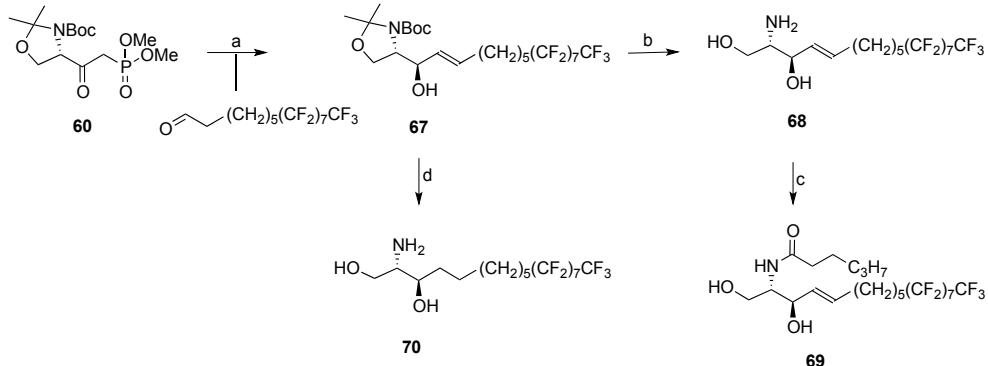
Reagent and conditions: (a) (i) K_2CO_3, H_2O, THF, 0 °C to r.t., 72 h, 79%; (ii) $Zn(BH_4)$, Et_2O, -78 to 0 °C, 88%; (b) 1 M HCl, THF, 70 °C, 18 h, 85%; (c) hexanoyl chloride, NaOAc, THF/H_2O, r.t., 12 h. 90%; (d) (i) Pd/C, H_2 (g), MeOH, r.t., 3 h; (ii) 1 M HCl, THF, reflux, 2 h, 63%.

1.9 Contents of this thesis

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

1.10 References

[1]   a) P. Nussbaumer, *ChemMedChem* **2008**, *3*, 543-551; b) C. R. Gault, L. M Obeid, Y. A. Hannun, *Adv. Exp. Med. Biol.* **2010**, *688*, 1-23.

[2]   K. C. Kopaczyk, N. S. Radin, *J. Lipid Res.* **1965**, *6*, 140-145.

[3]   A. K. Hajra, D. M. Bowen, Y. Kishimoto, N. S. Radin, *J. Lipid Res.* **1966**, *7*, 379-386.

[4]   B. Albrecht, G. Pohlentz, K. Sandhoff, G. Schwarzmann, *Chem. Phys. Lipids* **1997**, *86*, 37-50.

[5]   J. Kanfer, *J. Biol. Chem.* **1965**, *240*, 609-612.

[6]   G. R. Duffin, G. J. Ellames, S. Hartmann, J. M. Herbert, D. I. Smith, *J. Chem. Soc.*, *Perkin Trans. 1.* **2000**, 2237-2242.

[7]   W. W. Chen, A. B Moser, H. G. Moser, *Arch. Biochem. Biophys.* **1981**, *208*, 444-455.

[8]   N. W. Barton, A. Rosenberg, *J. Biol. Chem.* **1974**, *250*, 3966-3971.

[9]   Y. Barnholz, A. Roitman, S. Gatt, *J. Biol. Chem.* **1966**, *241*, 3731-3737.

[10]   G. Schwarzmann, *Biochim. Biophys. Acta* **1978**, *529*, 106-114.

[11]   D. E. Ong, R. N. Brady, *J. Biol. Chem.* **1973**, *248*, 3884-3888.

[12]   M. Iwamori, H. W. Moser, Y. Kishimoto, *J. Lipid Res*. **1975**, *16*, 332-336.

[13]   S. Li, J. Pang, W. K. Wilson, G. J. Schroepfer Jr., *J. Labelled Cpd. Radiopharm.* **1999**, *42*, 815-826.

[14]   M. C. McMaster Jr., N. S. Radin, *J. Labelled Comp. Radiopharm.* **1976**, *13*, 353-357.

[15]   Y. Suzuki, K. Suzuki, *J. Lipid Res.* **1972**, *13*, 687-690.

[16]   N. S. Radin, L. Hof, R. M. Bradley, R. O. Brady, *Brain Res.* **1969**, *14*, 497-505.

[17]   M. J. Brammer, *J. Neurosci.* **1984**, *42*, 135-141.

[18]   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**, 2661-2677.

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

[20]   T. Mehnert, K. Jacob. R. Bittman, K. Beyer, *Biophys. J.* **2006**, *90*, 939-946.

[21]   W. Stoffel, D. LeKim, T. S. Tschung, *Hoppe-Seyler's Z. Physiol. Chem.* **1971**, *352*, 1058-1064.

[22]   M. Koval, R. E. Pagano, *J. Cell Biol.* **1989**, *108*, 2169-2181.

[23]   N. G. Lipsky, R. E. Pagano, *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 2608-2612.

[24]   G. Schwarmann, M. Wendeler, K. Sandhoff, *Glycobiol.* **2005**, *15*, 1302-1311.

[25]   T. Hakogi, T. Shigenari, S. Katsumura, T. Sano, T. Kohno, Y. Igarashi, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 661-664.

[26]   Y. Liu, R. Bittman, *Chem. Phys. Lipid* **2006**, *142*, 58-69.

[27]   R. E. Pagano, O. C. Martin, H. C. Kang, R. P. Haugland, *J. Cell Biol.* **1991**, *113*, 1267-1279.

[29]   S.-H. Son, S. Daikoku, A. Ohtake, K. Suzuki, K. Kabayama, Y. Ito, O. Kanie, *Chem. Commun.* **2014**, *50*, 3010-3013.

[30]   I. I. Mikhalyov, J. G. Molotkovsky, *Russian J. Bioorg. Chem.* **2003**, *29*, 190-197.

[31]   R. Kim, K. Lou, M. L. Kraft, *J. Lipid Res.* **2013**, *54*, 265-275.

[32]   G. Schwarzmann, K. Sandhoff, *Methods Enzymol.* **1987**, *138*, 319-341.

[33]   J. G. Molotkovsky, I. I. A. B. Imbs, L. D. Bergelson, *Chem. Phys. Lipids* **1991**, *58*, 199-212.

[34]   M. C. Correa-Freire, Y. Barenholz, T. E. Thompson, *Biochem.* **1982**, *21*, 1244-1248.

[35]   R. C. Hresko, I. P. Sugar, Y. Barenholz, T. E. Thompson, *Biophys. J.* **1987**, *51*, 725-733.

[36]   P. Antes, G. Schwarzmann, K. Sandhoff, *Eur. J. Cell Biol.* **1992**, *59*, 27-36.

[37]   A. Dagan, V. Agmon, S. Gatt, T. Dinur, *Methods Enzymol.* **2000**, *312*, 293-304.

[38]   K. P. Bhabak, D. Proksch, S. Redmer, C. Arenz, *Bioorg. Med. Chem.* **2012**, *20*, 6154-6161.

[39] P. Ettmayer, A. Billich, T. Baumruker, D. Mechtcheriakova, H. Schmid, P. Nussbaumer, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1555-1558.

[40] P. Nussbaumer, P. Ettmayer, C. Peters, D. Rosenbeiger, K. Högenauer, *Chem. Commun.* **2005**, 5086-5087.

[41] C. Peters, A. Billich, M. Ghobrail. K. Högenauer, T. Ullrich, P. Nussbaumer, *J. Org. Chem.* **2007**, *72*, 1842-1845.

[42] C. Bedia, J. Casas, V. Garcia, T. Levada, G. Fabriàs, *ChemBioChem* **2007**, *8*, 642-648.

[43] M. Garrido, J. L. Abad, G. Fabriàs, J. Casas, A. Delgado, *ChemBioChem* **2015**, *16*, 641-650.

[44] M. Dauner, E. Batroff, V. Bachmann, C. R. Hauck, V. Wittman, *Bioconjug. Chem.* **2016**, *27*, 1624-1637.

[45] M. S. Sandbhor, J. A. Key, I. S. Strelkov, C. W. Cairo, *J. Org. Chem.* **2009**, *74*, 8669-8674.

[46] A. Gaebler, R. Milan, L. Straub, D. Hoelper, L. Kuerschner, C. Thiele, *J. Lipid Res.* **2013**, *54*, 2282-2290.

[47] A. Ohtaka, S. Daikoku, K. Suzuki, Y. Ito, O. Kanie, *Anal. Chem.* **2013**, *85*, 8475-8482.

[48] L. Elsen, R. Betz, G. Schwarmann, K. Sandhoff, G. van Echten-Deckert, *Neurochem. Res.* **2002**, *27*, 717-727.

[49] S. Schütze, M. Wickel, M. Heinrich, S. Winoto-Morbach, T. Weber, J. Brunner, M. Krönke, *Methods Enzymol.* **2000**, *312*, 429-438.

[50] P. Haberkant, O. Schmitt, F.-X Contrerea, C. Thiele, K. Hanada, H. Sprong, C. Reinhard, F. T. Weiland, B. Brügger, *J. Lipid Res.* **2008**, *49*, 251-262.

[51] P. Haberkant, F. Stein, D. Höglinger, M. J. Gerl, B. Brügger, P. P. Van Veldhoven, J. Krijgsveld, A.-C. Gavin, C. Schultz, *Chem. Biol.* **2016**, *11*, 222-230.

[52] M. Wendeler, J. Hoernschemeyer, D. Hoffmann, T. Kolter, G. Schwarzmann, K. Sandhoff, *Eur. J. Biochem.* **2004**, *271*, 614-627.

[53] S. Saito, Y. Murai, S. Usuki, M. Yoshida, M. A. S. Hammam, S. Mitsutake, K. Yuyama, Y. Igarashi, K. Monde, *Eur. J. Org. Chem.* **2017**, 1045-1051.